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## Separation and Purification Section: Summary of Activities July 1969 to June 1970

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# TECHNICAL NOTE 549

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## Separation and Purification Section:

### Summary of Activities - July 1969 to June 1970

David H. Freeman and Walter L. Zielinski, Jr.  
Editors

Separation and Purification Section  
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## FOREWORD

The Analytical Chemistry Division was established as a separate division at the National Bureau of Standards on September 1, 1963, and became part of the Institute for Materials Research in the February 1, 1964, reorganization. It consists at present of nine sections and about 100 technical personnel encompassing some 60 different analytical competences from activation analysis and atomic absorption to vacuum fusion and x-ray spectroscopy. These competences, and in turn the sections which they comprise, are charged with research at the forefront of analysis as well as awareness of the practical sample, be it standard reference material or service analysis. In addition it is their responsibility to inform others of their efforts.

Formal publication in scientific periodicals is a highly important output of our laboratories. In addition, however, it has been our experience that informal, annual summaries of progress describing efforts of the past year can be very valuable in disseminating information about our programs. A word is perhaps in order about the philosophy of these yearly progress reports. In any research program a large amount of information is obtained and techniques developed which never find their way into the literature. This includes the "negative results" which are so disappointing and unspectacular but which can often save others considerable work. Of importance also are the numerous small items which are often explored in a few days and which are not important enough to warrant publication--yet can be of great interest and use to specialists in a given area. Finally, there are the experimental techniques and procedures, the designs and modifications of equipment, etc., which often require months to perfect and yet all too often must be covered in only a line or two of a journal article.

Thus our progress reports endeavor to present this information which we have struggled to obtain and which we

feel might be of some help to others. Certain areas which it appears will not be treated fully in regular publications are considered in some detail here. Other results which are being written up for publication in the journal literature are covered in a much more abbreviated form.

At the National Bureau of Standards, publications such as these fit logically into the category of a Technical Note. We plan to issue these summaries for all of our sections. The following is the fourth annual report on progress of the Separation and Purification Section.

W. Wayne Meinke, Chief  
Analytical Chemistry Division

## PREFACE

This is the fourth annual report on the activities of the Separation and Purification Section. The work of the Section is devoted to those problems of science and measurement in the separations field that seem most significant in terms of the larger goals of the National Bureau of Standards. Energy is divided between the long and short range, the basic and the applied, and between the abstract need for the related supportive knowledge and a more direct need for constructive accomplishment.

Three areas of interest have dominated the past year. First, particle metrology has received attention in reply to the growing awareness of need. The rules for characterizing a particulate sample are incomplete and this section's experience with ion exchange microstandards is suitable for refocussing into a more general context. Second, liquid chromatography is receiving a general and strong resurgence of interest because of the recognition that recent instrumental developments have opened vast fields for extended interest and utility. This is somewhat of a difficult area experimentally and the section is dedicating part of its effort toward creating simplifications for others to use. Third, there is a widespread and basic need for improved guidelines on the principles and practices that should be used in preparing, characterizing and containing chemical reagents of high quality. To illustrate, the current general interest in supplying quality organic chemical reagents is, at best, without pride. Some authorities seem to encourage a lack of common sense in carrying out elementary practices in these areas. Common to all of the above is our constant finding of many problems for concern in the separation and purification field and there is a growing significance attached to the need to be as alert as possible in trying to channel our energies as effectively as possible.

It has been necessary occasionally to identify commercial materials and equipment in this report. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

David H. Freeman, Chief  
Separation & Purification Section

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SEPARATION AND PURIFICATION SECTION: SUMMARY OF ACTIVITIES  
JULY 1969 to JUNE 1970

Edited by David H. Freeman and  
Walter L. Zielinski, Jr.

This represents the report of activities for the Separation and Purification Section for fiscal year 1970. Studies on the properties of ion exchange Standard Reference Material microbeads is extended into the effect of humidity on bead volume, thermal effects on bead stability, and measurement of ion exchanger capacity. Zeolitic particles are evaluated for their potential as ion exchange microstandards. Preliminary investigations are completed for the quantitative infrared measurement of crosslinking in poly (styrene/divinylbenzene) copolymers. Light and electron microscopy are used to help reveal the heterogeneity in a test dust standard. Progress is reported in the development and understanding of analytical liquid chromatography with an increased direction toward gel chromatography. The selection of apparatus components, their performance, and their application to examination of the clinical Standard Reference Material, bilirubin, is described. Particle contamination in inorganic salts and in container walls are examined, the former by ultrafiltration and accurate light scattering measurements and the latter by microscopic methods.

Key words: Air pollution; bilirubin; infrared analysis; ion exchange; liquid chromatography; purification; separation; styrene/divinylbenzene; zeolites.

## 1. CHEMICAL MICROSTANDARDS

### A. Introduction

In our last annual report [1] we described the principles, technique and the associated requirements for the preparation of single bead ion exchange microstandards containing a known minute weight of a given metal ion. In this report we cited

the availability of the first "encapsulated" metal ion standard, that of calcium (SRM 1800), on single cation exchange beads, containing from nanogram to 10 picograms of calcium with a certified accuracy of within 10 percent. A detailed summary describing the rationale, preparation and analytical calibration of ion exchange bead microstandards has recently been published [2]. The availability of such standards will serve to pique the interest of investigators requiring small accurate amounts of a given cation (or cation mixtures) in their research studies. They have direct application to the calibration of instruments employed in trace metal ion measurement; viz., neutron activation, the mass spectrometer, and the electron or laser microprobe. During the past year, Pb(II), Cu(II), Li, Cr(III), Na, Ni(III), Fe(III), Mg(II), Zn(II), and U(IV) have been loaded onto ion exchange beads. The difficulties associated with Hg(II) loaded beads will be cited below in Section I.D.

#### B. Nanogram Standards

We had previously described the problems involved in obtaining quantitative desorption of certain multivalent cations (eg. Cr(III)) from cation exchange beads [1]. The difficulties related to Cr(III) stripping have been resolved in the past year. The details of these investigations have been submitted for publication. In brief, it was found that Cr(III) is removed from loaded beads with 98 percent recovery as chromate in 30 minutes using a 1:4 mixture of 1N NaOH:50% H<sub>2</sub>O<sub>2</sub> at pH 7.2 at 30 °C. This result is compared in Figure 1 with three other less effective procedures.

#### C. Microgram Standards

In order to extend the cation weight range to the microgram region, homogeneous, spherical ion exchange beads having particle diameters of 100-200  $\mu\text{m}$  need be employed (Figure 2). Such beads are currently undergoing characterization to provide

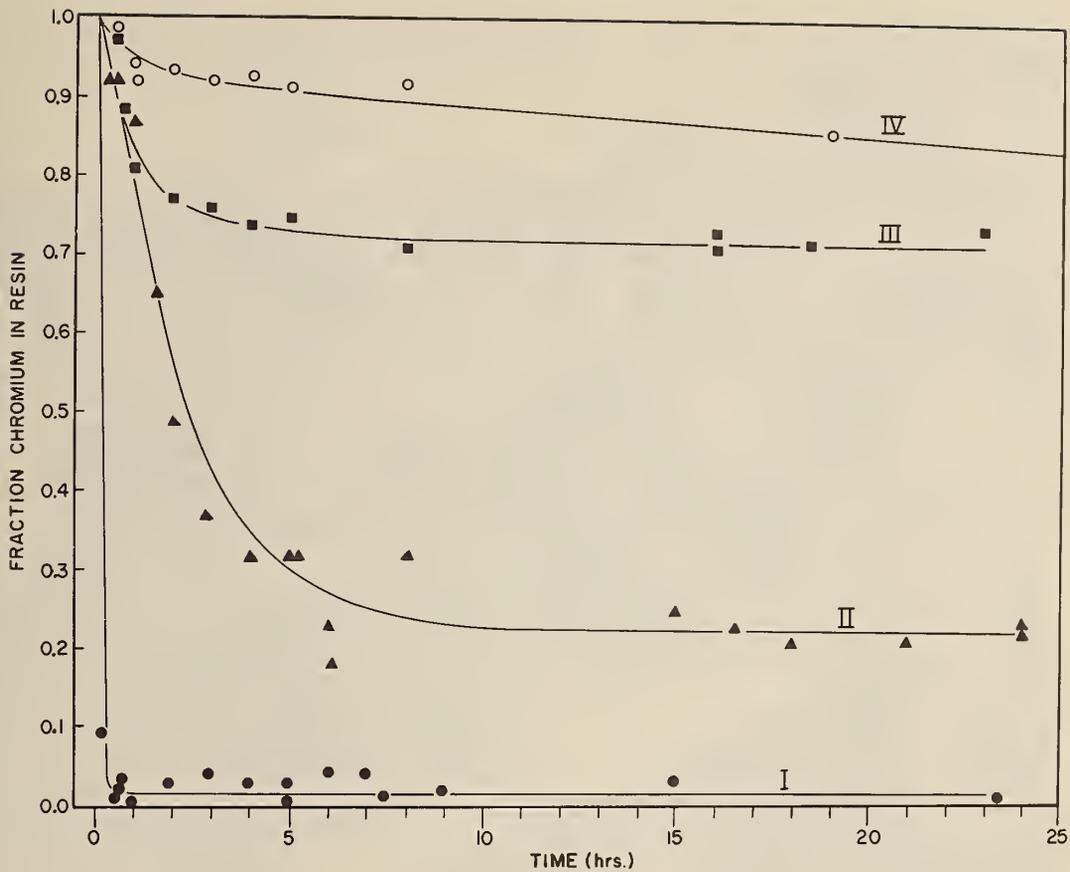


Figure 1. Counterion stripping of Cr(III) exchange beads.

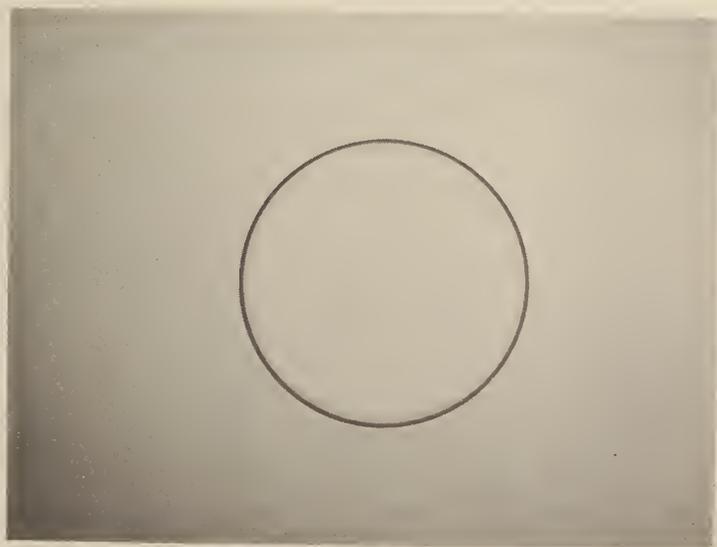


Figure 2. Exchange beads for microgram standards.

a working matrix for lead microstandards in the range of one microgram and smaller.

#### D. Thermal Stability

The thermal stability of several batches of ion exchange beads loaded with different cations was examined in vacuo ( $10^{-6}$   $\mu\text{m Hg}$ ) from 40-360 °C, using the solid probe of a quadrupole mass spectrometer. The only loss of metal ion occurred with Hg(II) loaded beads. Here, traces of Hg vapor were detected in the ion chamber as low as 60 °C, increasing significantly from 80-360 °C. Similar effects were noted for Hg(II) loaded zeolite particles.

#### E. Humidity Effects upon Ion Exchange Bead Diameters

The dependence of the swollen volume of an ion exchange resin upon humidity is believed to be a thermodynamic property and, therefore, path independent. The testing of this hypothesis has not been thoroughly examined beyond the initial studies of Boyd and Soldano [3] who first established experimental consistency with isopiestic water vapor pressure equilibrations of various ion exchangers. At water vapor pressures corresponding to water activities of less than 0.2, slower swelling or deswelling rates are expected. In addition, there is a possibility that repeated swelling cycles towards higher humidities could be influenced by resin structural changes.

The experimental approach used in the present effort is based upon the utility of microscopy to measure the diameters of individual ion exchange beads, and the use of recirculated air that is bubbled through aqueous glycerol media in order to fix the humidity level. The constant temperature humidification apparatus is shown in Figure 3. The microscope slide unit is simply a conventional cell that is sealed with the beads inside, and with ports at opposite lateral ends to permit entry and exit of the circulated vapor. The air pump

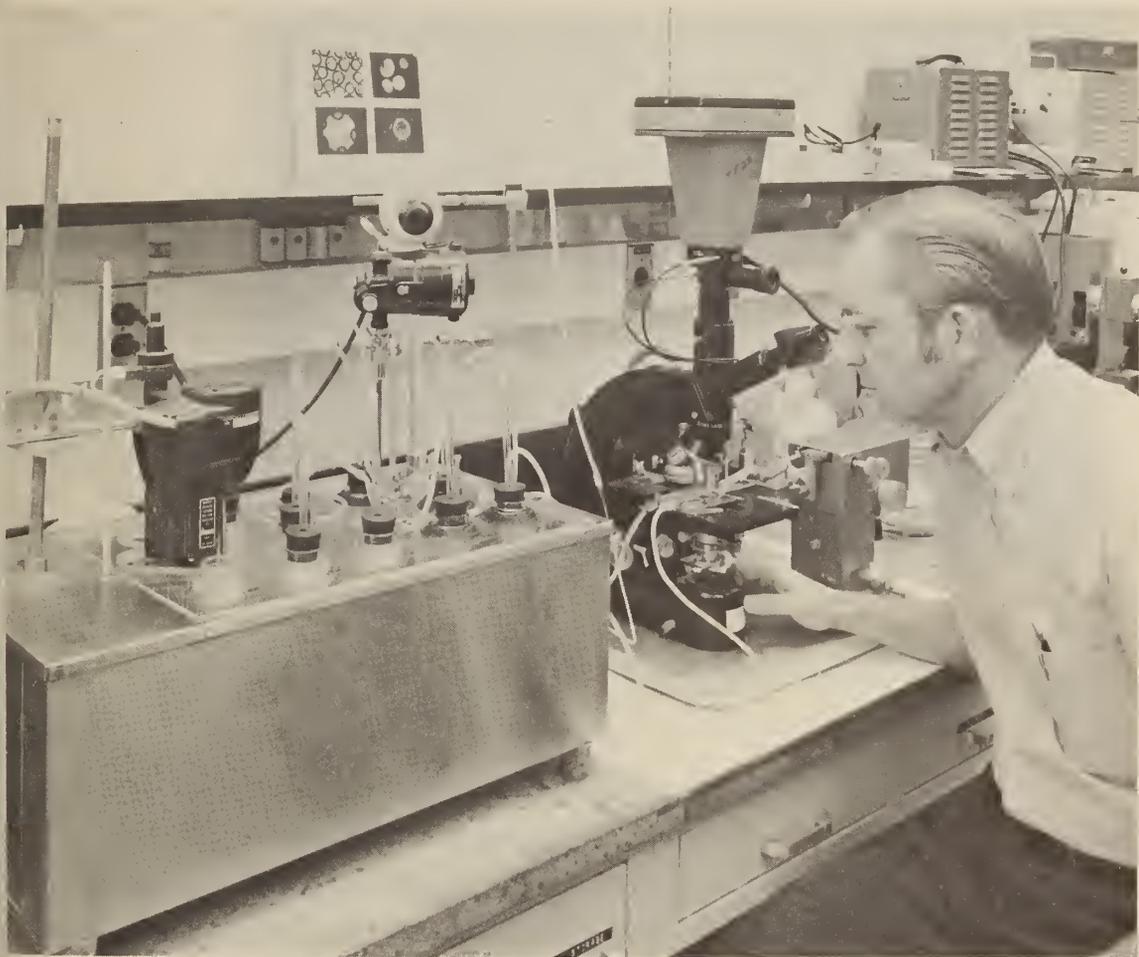


Figure 3. System for relative humidity studies.

is simply that used in an ordinary fish tank. The air follows the simple circle: pump/glycerol-water/cell. The cell is shown in Figure 4.

A constant procedure was followed with sodium form cation exchanger beads (8 percent DVB) that were being independently studied for their potential value as microstandards having counterion mass up to one microgram. The humidification procedure involved placing five or six beads in the cell, cementing on a top cover slip, and allowing the humidified air to circulate for a period of at least 24 hours.

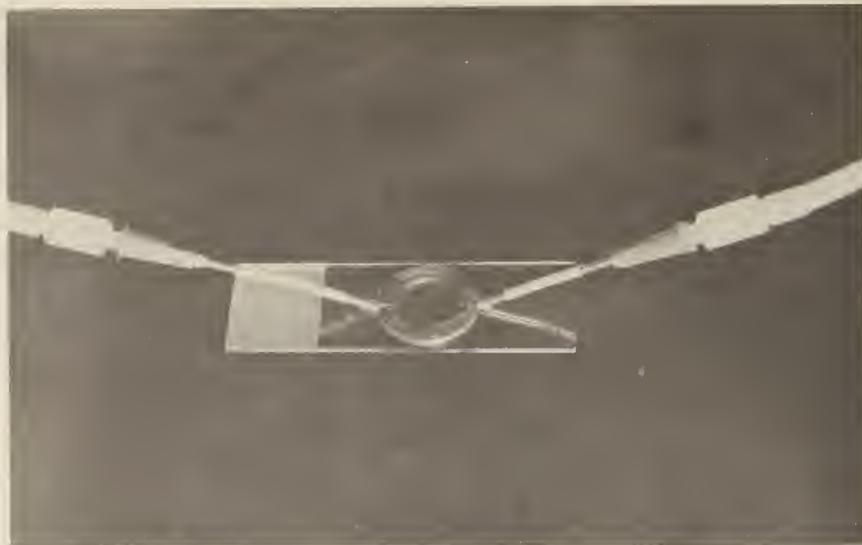


Figure 4. Humidity cell.

The result of six repeated swelling cycles between water activities of 0.5 and 0.1 showed volume ratios (swollen/unswollen beads) of  $1.139 \pm 0.002$  where the indicated standard deviation for six beads (69 bead diameter measurements, in toto) is about that which would be obtained due to measurement error alone. In a similar experiment, the cycling was between 0.8 and zero water activity, the latter being obtained after careful vacuum oven drying at 130 °C overnight. A larger variation was found here in the volume ratio  $1.499 \pm 0.019$  for five beads and six swelling cycles (55 diameter measurements). The error increase seems to be associated with measurement of the state at increased humidities. To illustrate, the relative standard deviation of reproducing a bead volume at water activity 0.8 was significantly higher (value of 0.007) than the value at a lower water activity (which ranged from 0.003 to 0.005). It is possible that the highest water activity of 0.8 involved coating the bead with a water film, which would be consistent with the observations of an apparently higher surface reflectivity (seen but not measured).

Finally, six beads were taken through an excursion from the dry state along the series of water activity: viz: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.6, 0.4, 0.2, and 0.1. Reproducibility was consistently apparent with relative standard deviations that did not exceed 0.6 percent, except for one point at 0.4 activity. The averaged results are shown in Figure 5. The end point at unit water activity was obtained by measuring the usual swelling ratio in water (total immersion). It is noteworthy that the results of low water activities do show a trend headed above an intercept with the zeros origin.

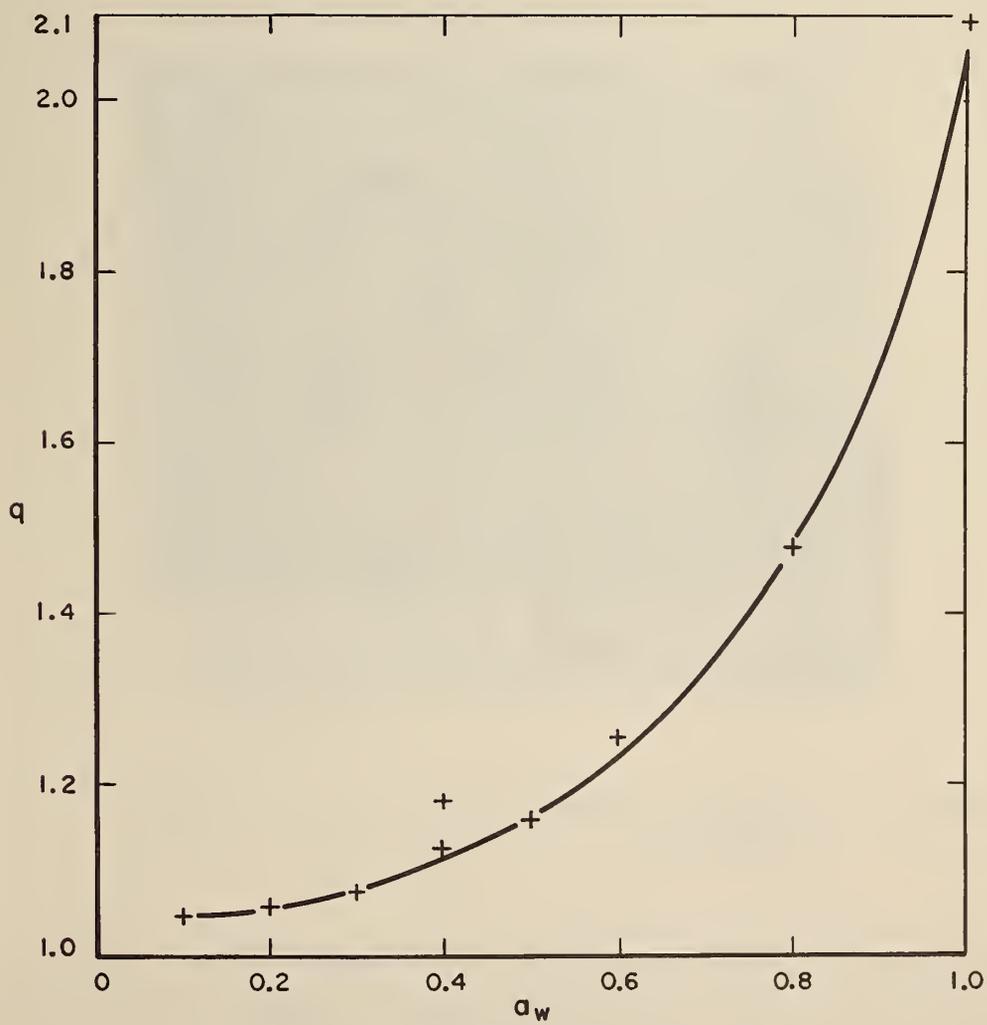


Figure 5. Exchange bead volume ratios as a function of water activity in humidified air.

This agrees with the corresponding isopiestic weight measurements reported by Boyd and Soldano [3].

F. Zeolites

Some of the disadvantages inherent in organic ion exchange beads, such as thermal instability, or the variation of bead volume with water content, might be overcome by using inorganic ion exchange materials. Zeolites are obvious candidates for study. Their aluminosilicate framework affords a chemically well-defined structure which retains its external shape independent of water content. The potential drawback to the use of zeolitic particles in microstandard work is that they often possess irregular shapes (Figure 6). Certain synthetic

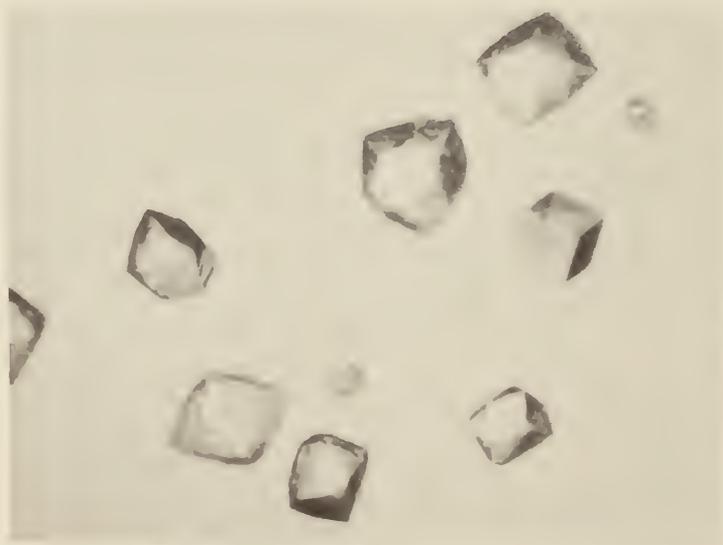


Figure 6. Photomicrograph of irregular zeolite particle.

zeolites, however, are available, at least in part, in regular geometric forms, and we have obtained octahedral and cubic particles of synthetic fajasite from Dr. Howard Sherry of Mobil Research and Development Corporation, Princeton, New Jersey.

The chemical homogeneity of the preferable cubic geometry was evaluated by neutron activation at the NBS reactor, followed by gamma radiation counting of  $^{24}\text{Na}$  decay and calculation for sodium content. The particle volume was determined by measurement of at least two sides of each cubic particle using a light microscope (Figure 7). The precisely linear relationship between sodium content and volume of single particles is shown in Figure 8. Hence, it should be possible to use such zeolitic particles as calibration standards for sodium, directly. This work is being prepared for publication.



Figure 7. Photomicrograph of a cubic zeolite particle.

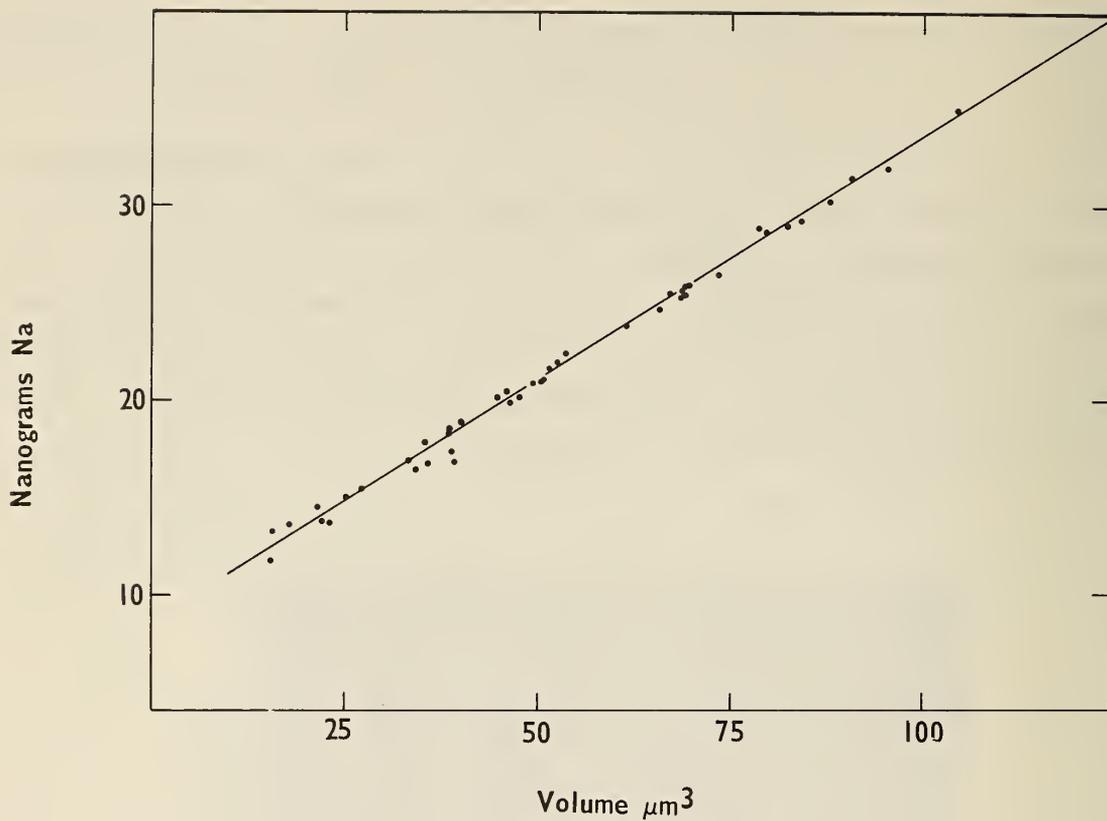


Figure 8. Linear relationship of sodium content as a function of particle volume in cubic zeolites.

## 2. ION EXCHANGE NETWORKS

### A. Capacity Measurements

The standard procedure employed for the determination of the capacity (i.e., the concentration of available exchange sites) of an ion exchanger involves titration of the hydrogen counterion form of the exchanger with a standardized strong basic titrant (eg. NaOH). The capacity value (meq/g) is then obtained from the relationship

$$C = \frac{\text{meq NaOH}}{\text{g exchanger}}$$

where C is the capacity of the dry exchanger expressed in the sodium form ( $R^-Na^+$ ). Best results are obtained if the exchanger is allowed to stand overnight in excess base, followed by back titration in the morning with standardized acid. Precautions were taken to insure that alkali consumption by reaction with atmospheric  $CO_2$ , or with the container walls, was negligible. The apparatus setup is shown in Figure 9. The precision of the method was found to be excellent ( $4.580 \pm 0.005$  for three replicates of a highly sulfonated sample). Some capacity values determined for samples containing varying degrees of sulfonation are given in Table 1.

Since the capacity of sulfonated poly(styrene/divinylbenzene) exchangers is related to the concentration of available ion exchange sites, and hence to the concentration of sulfonic acid groups in the exchanger, it is expected that a relationship might exist between the degree of absorption of infrared light (at a frequency attributable to the  $-SO_3^-$  group) and the capacity value, for such exchangers. Such a relationship does, in fact, exist. Quantitative measurements were made of the ratios of the depth of the absorbance band at  $1408\text{ cm}^{-1}$  (attributable to sulfonation), to the depth of the absorbance band at  $1450\text{ cm}^{-1}$  (copolymer skeleton). Figure 10 shows the



Figure 9. Apparatus for measurement of ion exchanger capacity.

position of these bands in the spectrum. The comparison of the infrared absorption ratio to the titrated capacity is given in Figure 11.

Table 1. Capacity values of exchangers having varied degrees of sulfonation.

| Sample <sup>a</sup><br>type | Duration of sulfonation (hours) |       |       |
|-----------------------------|---------------------------------|-------|-------|
|                             | 5                               | 25    | 125   |
| 1                           | 0.000                           | 0.141 | 3.121 |
| 2                           | 0.000                           | 0.175 | 3.530 |
| 3                           | 2.320                           | 2.515 | 2.399 |
| 4                           | 2.756                           | 3.035 | 2.876 |
| 5                           | 3.122                           | 3.430 | 3.202 |
| 6                           | 3.334                           | 3.652 | 3.490 |

<sup>a</sup>Samples were sulfonated using varied weight ratios of nitrobenzene:H<sub>2</sub>SO<sub>4</sub>:copolymer.

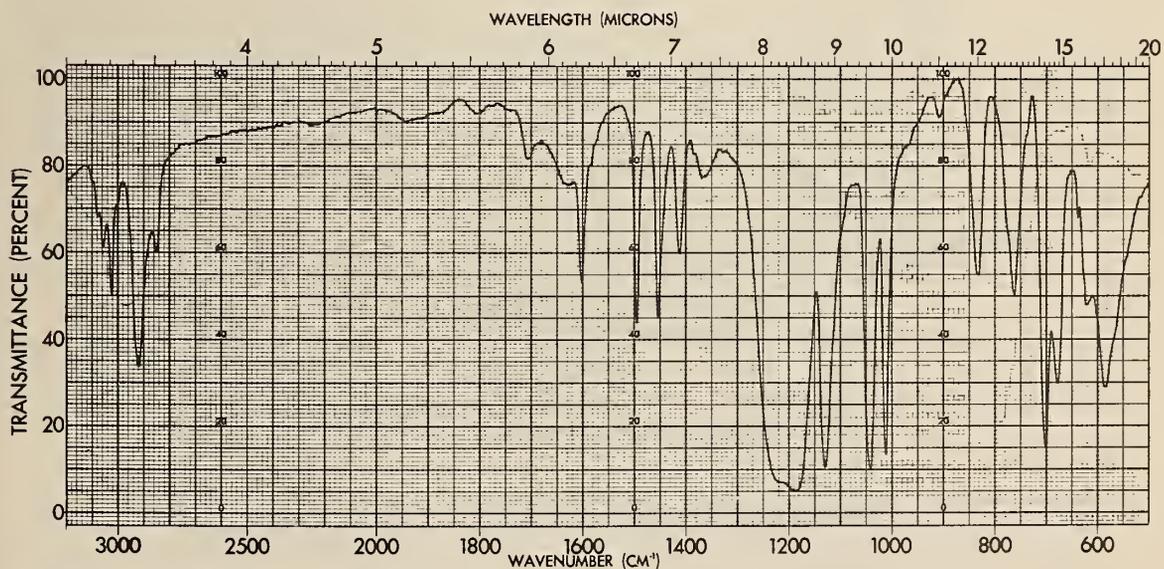


Figure 10. Infrared spectrum of sulfonated poly (styrene/DVB).

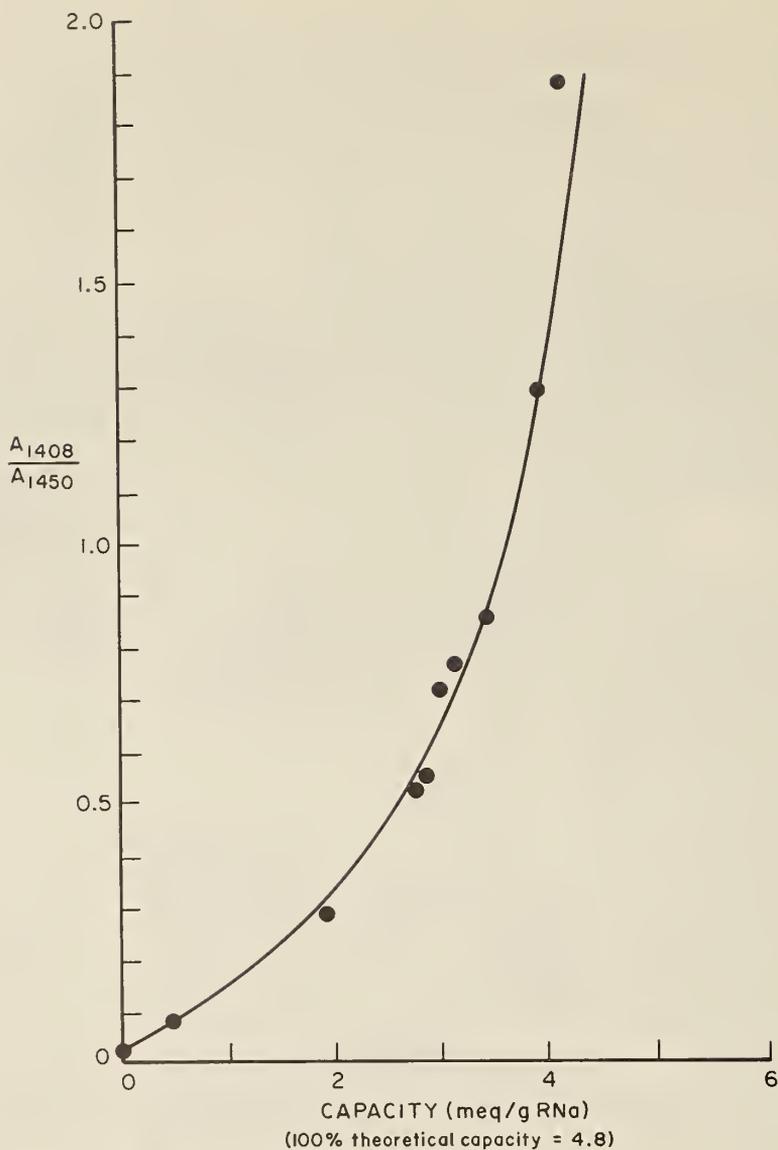


Figure 11. Correlation of infrared absorbance to titrated capacity.

#### B. Network Characterization

The total characterization of a copolymer matrix requires a knowledge of the intricacies of the topology of the cross-linked molecular strands, and a knowledge of the chemical composition composing the copolymer bead. The experimental measurement of the latter is possible by combined gas chromatography (to determine the molar composition of the divinylbenzene crosslinking reagent) and infrared spectrophotometry

(to measure the molar concentration of the divinylbenzene isomers in the copolymer proper). The location of the vibrational energies in the infrared spectrum for meta- and para-divinylbenzene crosslinking in copoly(styrene/DVB) was reported last year [1]. During the past year, the quantitative estimation of crosslinking has been worked out [4].

The procedure is based upon the determination of standard curves for each type of crosslinking using styrene copolymers prepared separately with the single DVB isomers. The standard curves plot the absorbance ratio of the DVB isomer absorption to that of a reference band attributable to the styrene chain, as a function of DVB isomer concentration (expressed as  $X_1/(1-X_1)$ , where  $X_1$  is the mole fraction of DVB isomer used in the styrene-DVB reagent mixture). The relationships were found to follow Beer's law linearity through  $0.08 X_1$  of either DVB isomer. The standard curves are shown in Figure 12.

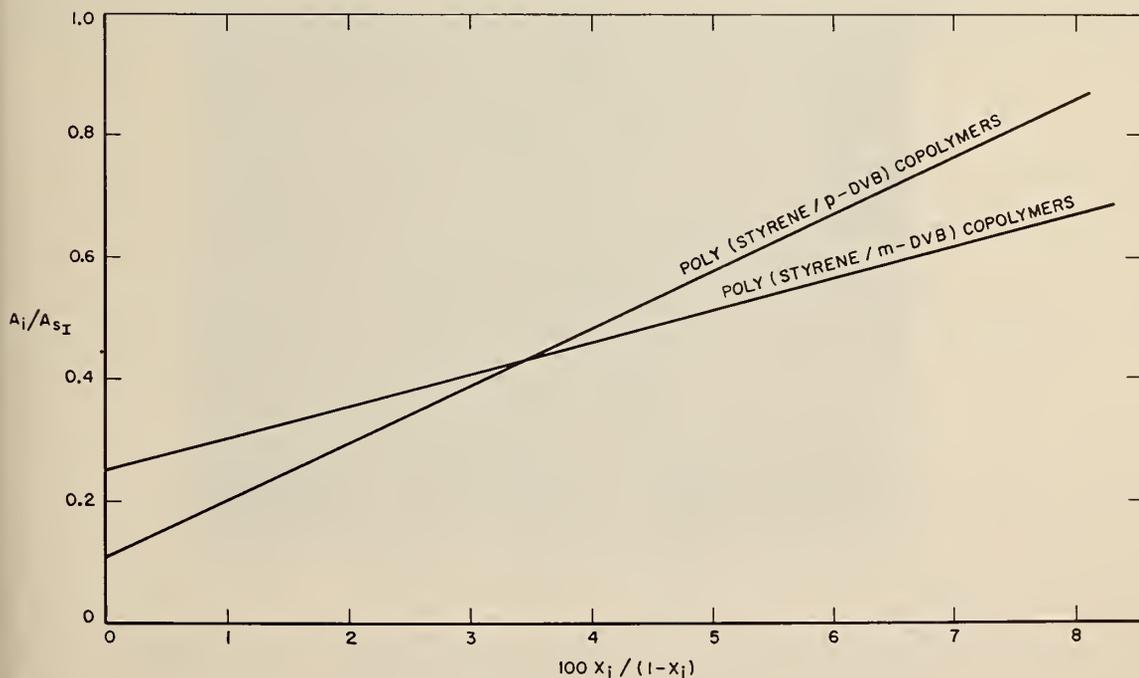


Figure 12. Relative absorbance as a function of DVB isomer crosslinking in poly(styrene/DVB) copolymers.

This study was extended to the infrared spectra of poly-(styrene/DVB) prepared with a crude DVB reagent that is commonly used in the commercial preparation of many copolymer networks. The method requires the use of the standard curves cited above, together with a knowledge of the original vinyl monomer composition of the technical DVB mixture. The latter information is readily obtainable by GLC analysis using a nematogenic liquid crystal stationary phase [1,5]. With this procedure in hand, quantitative measurements of the moieties of the meta and para isomers of ethylvinylbenzene and divinylbenzene in the copolymer are attainable [4].

### 3. MICROSCOPY

In the past year, the microscopy facility in our Section became involved in the examination of particles of 1  $\mu\text{m}$  or less. Some of the more important recent undertakings, for example, include the examination of (1) very fine quartz spheres, (2) a fine test dust sample, and (3) the particle contamination of cleaned microscope slides.

#### A. Quartz Spheres

The scrutiny of quartz spheres was undertaken primarily to obtain information on their sphericity and size range. The material was dispersed in cyclohexane by ultrasonic agitation and then placed on a glass slide. Using light microscopy, the best results were obtained by a 50X objective, with the sample exposed to air. The photomicrograph (625X) in Figure 13,



→ ← 10  $\mu\text{m}$

Figure 13. Photomicrograph of quartz spheres taken from a light microscope.

however, does not yield the desired information. One drop of the prepared sample was then placed on a formvar-carbon coated electron microscope grid and examined under the electron microscope. Figure 14 is an electron micrograph (29,000X) of

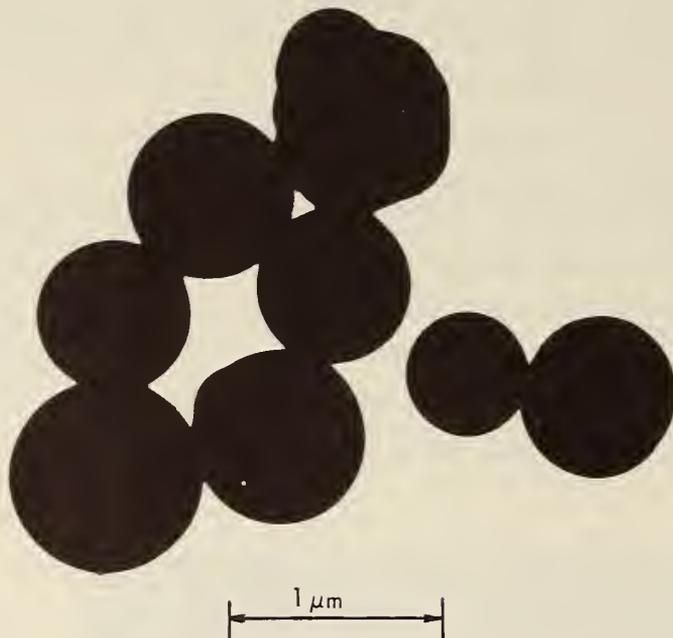


Figure 14. Electron micrograph of quartz spheres.

this material in which the particle diameters measured ranged from 0.91  $\mu\text{m}$  to 0.56  $\mu\text{m}$ . As indicated in this figure, the sphericity of these particles was found to be excellent.

Figure 15 is a photograph of the electron microscope which allows direct observations to 80,000X and is equipped with 35 mm roll film and 2 1/4 in x 2 1/4 in. plate cameras for electron micrography. Also shown in Figure 15 is the vacuum evaporator which is used for carbon coating films on specimen grids and metal shadowing of carbon replicas of materials that are not suitable for direct examination in the electron microscope.

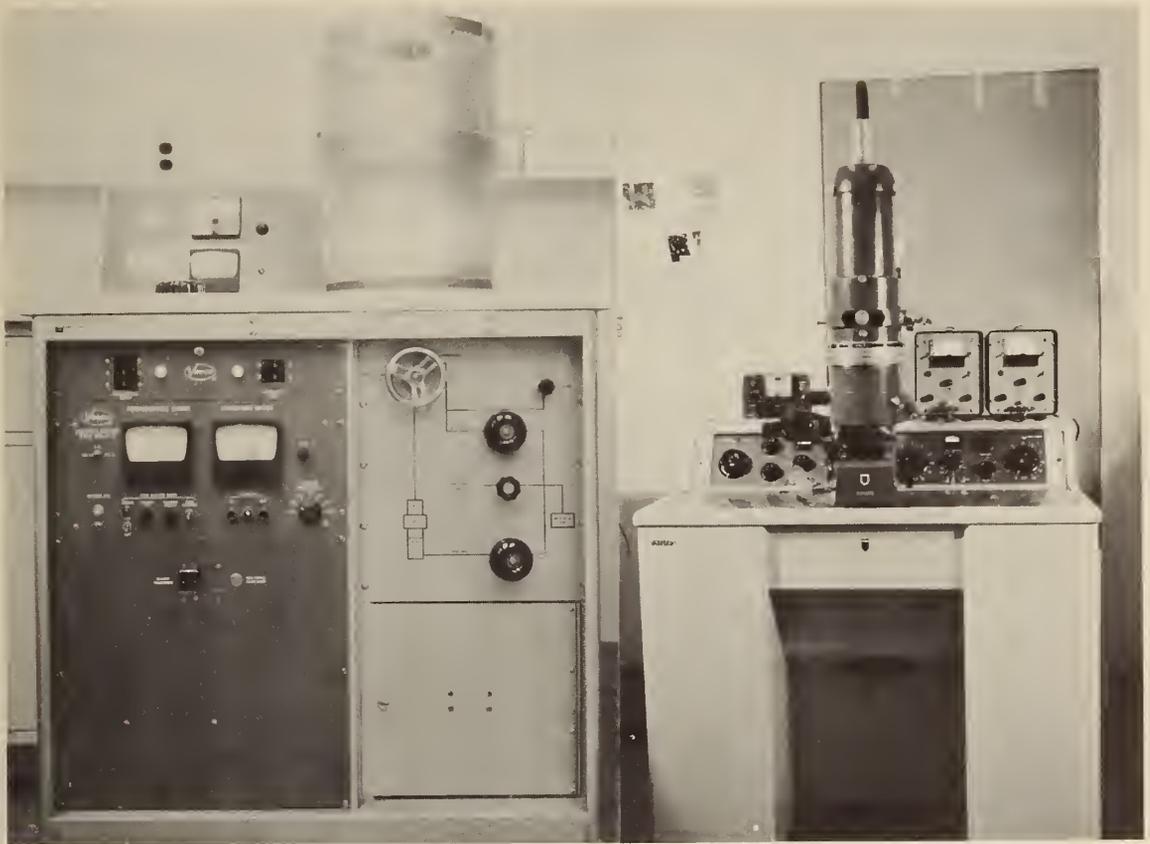


Figure 15. Electron microscope and vacuum evaporator.

B. Fine Test Dust

In October of 1969 we received a sample for evaluation identified as "AC Fine Test Dust." This material has been considered as a standard particulate contaminant. It is important, but likewise difficult, to measure trace particulate contamination of gaseous or liquid systems. New standards are required for the development of procedures which determine the effect of the contamination. A prime requirement of any material standard is chemical and physical homogeneity. Our evaluation of the material indicated sufficient heterogeneity so that its potential as a standard is somewhat marginal. Because of the growing need for this type of standard, the results of the study are given in the following.

The first indication of heterogeneity was provided with the use of conventional light microscopy. The photomicrographs in Figure 16 were obtained with the same dispersion of particles from the test sample. These photomicrographs show a mixture of opaque and transparent particles. Electron microscopy at magnifications at 28,700 showed that the particle size range extended to below  $10^{-2}$   $\mu\text{m}$ . See Figure 17.

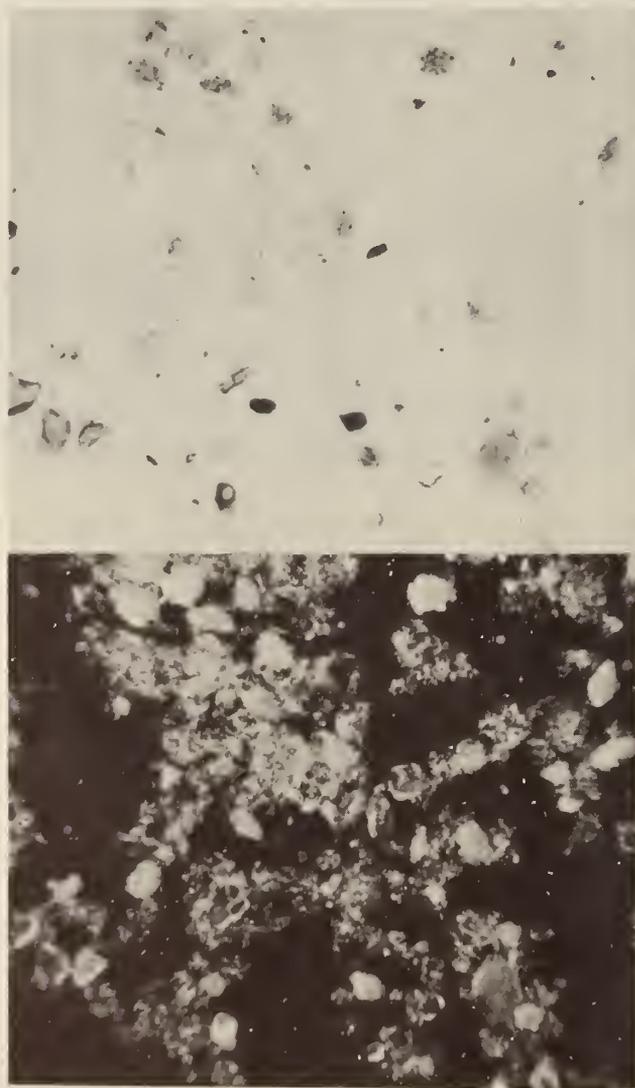


Figure 16. Photomicrograph of fine test dust taken from a light microscope with bright and dark field illumination.

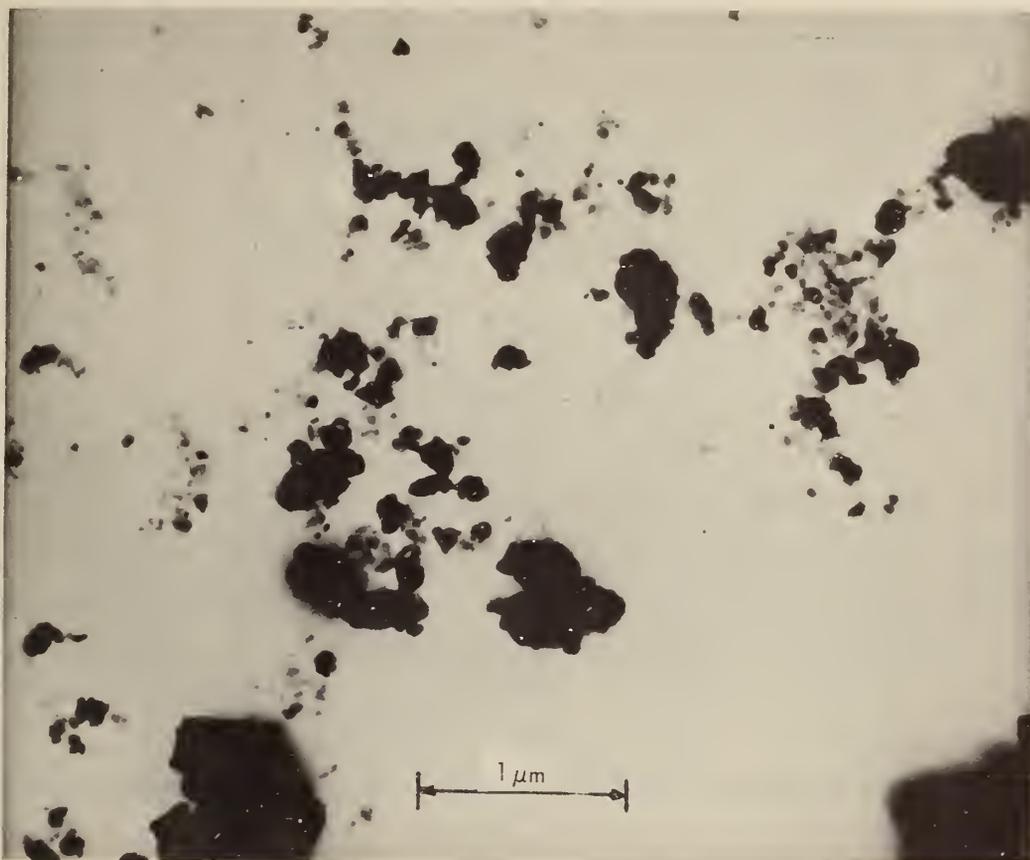


Figure 17. Electron micrograph of fine test dust.

A study of the particle size distribution was carried out using both electron and light microscopy with measurements of particle size (longest dimension) with the help of a Zeiss TGZ-3 particle size analyzer. The results are shown in Figure 18 where a differential histogram shows the apparently bimodal distribution which peaks at 0.6 and at 2  $\mu\text{m}$ . A very serious limitation to measurement accuracy is involved in view of the cross calibration between the light and the electron microscopy in the overlapping regions in the vicinity of 0.5 to 1  $\mu\text{m}$ . Although it was not carried out, it should be possible to scale the relative contributions of the two sized modes using a 5  $\mu\text{m}$  sieve.

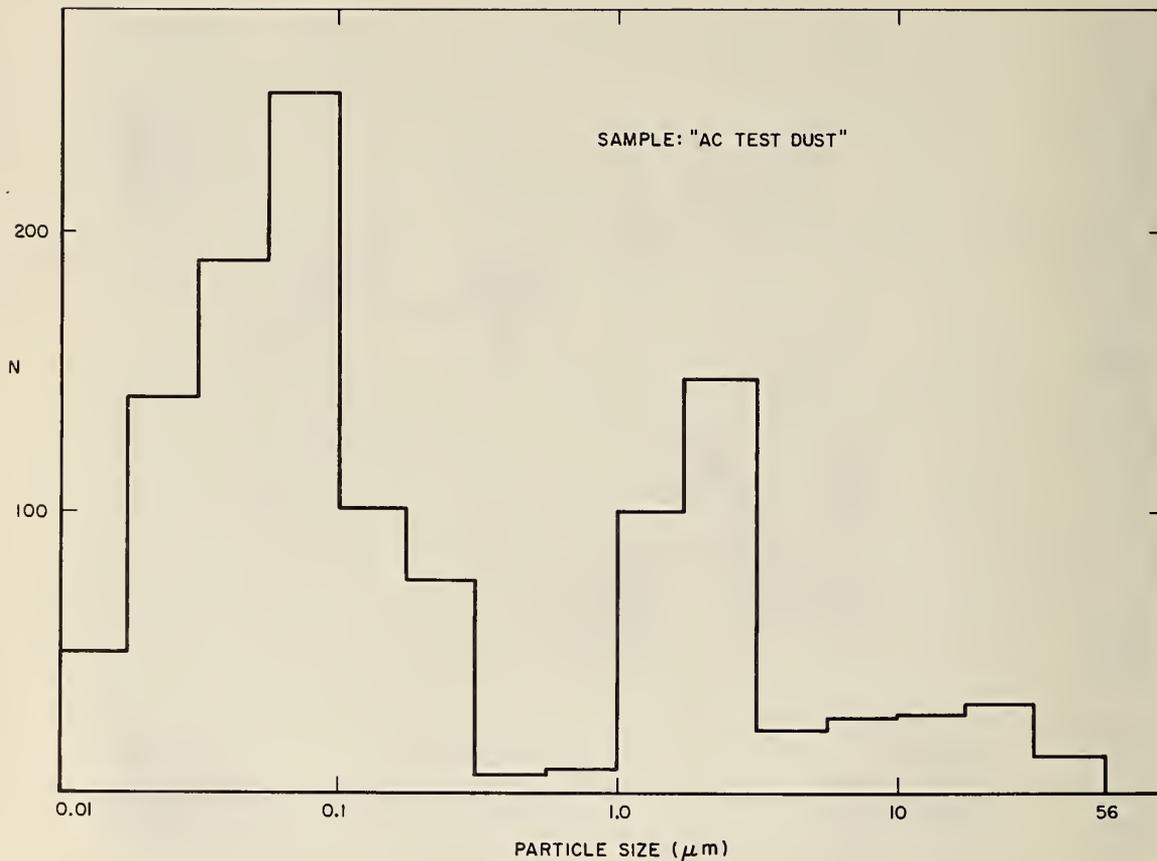


Figure 18. Histogram of AC test dust.

The density of the AC Test Dust sample was measured in a Linderstrom-Lang density gradient column with the use of ethylene dibromide and bromoform. The average density of the sample was near  $2.8 \text{ g/cm}^3$ , although this is only a rough estimate. Approximately 99 percent of the particles float on bromoform ( $2.9 \text{ g/cm}^3$ ). The remainder is composed of black particles of higher density. These are easily removed by centrifugation. A magnet placed into contact with the suspended sample will remove the heavier magnetic particles whose composition includes iron as a major constituent.

A qualitative analysis of AC Fine Test Dust by emission spectroscopy was carried out for us by Mrs. Virginia Stewart of the Spectrochemical Analysis Section. Major elements

include Al, Ca, Fe, Na, K and Si. The minor elements include Ag, Ba, Cr, Co, Mg, Mn, Ni, Pb, Sr, Ti, V and Zn.

An x-ray diffraction examination was carried out by Howard E. Swanson of the Crystallography Section at NBS. The results showed that the sample was composed primarily of alpha quartz and orthoclase feldspar (K, Al,  $\text{Si}_3\text{O}_8$ ) in approximately equal amounts. A small percentage of mica was also noted (perhaps 5 percent). These findings approximate the analysis of a powdered alkali granite or rhyolite, without appreciable chemical weathering.

The conclusion is that this sample is heterogeneous in several ways. First, there is wide distribution in opacity, density, and composition. The bimodal particle size distribution creates a serious impediment to the achievement of a meaningful size frequency analysis. The presence of magnetic particles represent an obvious opportunity for biased calibration of metallic equipment to which the magnetic particles would be attracted.

Recommendations for other possible materials that would offer better properties as a standard would be well guided on the basis of taking a material with well defined physical and chemical properties. Various geologically occurring materials can be prepared synthetically, thus minimizing the problems of heterogeneity which seem to be associated with the present sample.

### C. Cleaning of Microscope Slides

The direct use of microscope slides which have not been immaculately precleaned is unsuitable for the preparation of slides of chemical microstandards. The slide cleaning procedure used by our laboratory is illustrated by the photomicrographs in Figure 19. Dark-field transmitted light microscopy was used for the micrographs shown in this figure. Micrographs #1692 (250X) and #1693 (500X) illustrate a contaminated slide that was supposed to be clean. Micrograph #1709 (500X) shows the

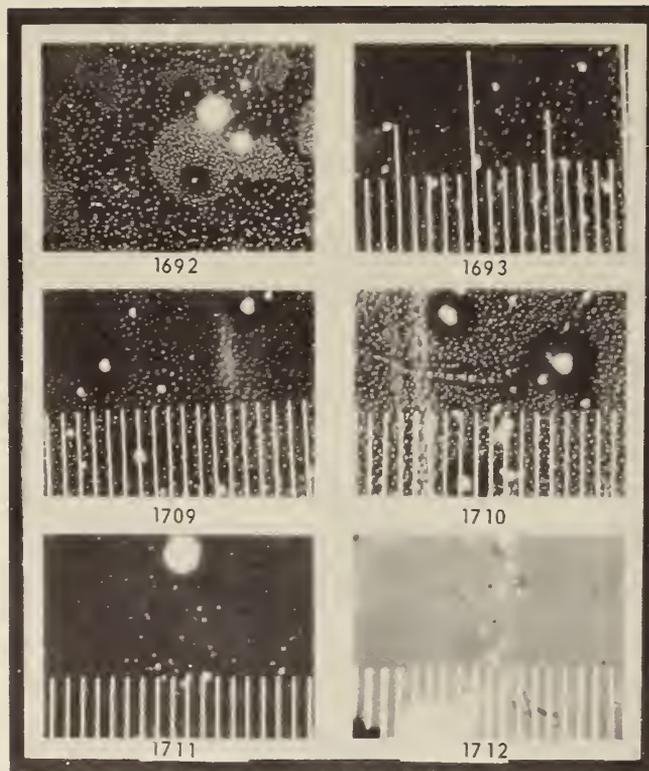


Figure 19. Cleaning of microscope slides.

surface of commercially available "precleaned" glass slides, while #1710 (500X) shows the same slide after application of cyclohexane followed by evaporation to dryness. Numbers 1711 and 1712 (500X) are two slides which have undergone rigorous precleaning with boiling  $\text{HNO}_3$  and copious rinses in distilled water with samples cast on them by our usual techniques. This work was carried out in a clean room facility. The lines on the micrographs are 10  $\mu\text{m}$  spacings of a stage micrometer obtained by double exposure of the micrograph.

## 4. CHROMATOGRAPHY

### A. Gas Chromatography of Aged Divinylbenzene

It was of interest to our studies on divinylbenzene reagents to ascertain the composition of two samples of meta-DVB, stored for over a year in a cold room maintained at 5 °C. The procedure for the complete resolution of the meta and para isomers of DVB on a nematogenic liquid crystal stationary phase has recently been published [5]. The internal standard procedure was used to determine the level of non-volatile "polymeric" material present in the sample (eg. [6]). The results are given in Table 2.

Table 2. Analysis of aged divinylbenzene by GLC.

| Sample No. | Relative percent in sample <sup>a</sup> |              |              |              |                    |                |
|------------|---|--------------|--------------|--------------|--------------------|----------------|
|            | <u>m-EVB</u>                            | <u>p-EVB</u> | <u>m-DVB</u> | <u>p-DVB</u> | <u>naphthalene</u> | <u>polymer</u> |
| 1          | 2.1                                     | ---          | 86.0         | 1.2          | ---                | 10.7           |
| 2          | 1.8                                     | 2.0          | 81.3         | 4.0          | 1.1                | 9.8            |

<sup>a</sup>Internal standard was n-tetradecane. Column: 0.125" o.d. by 10' s.s., packed with 9% 4,4'-dihexoxyazoxybenzene on 100/120 mesh AW-DMCS Chromosorb W (w/w). Temperatures: column 110 °C; injection port 180 °C; flame detector 210 °C. Carrier: 39 cm<sup>3</sup>/min He.

### B. Liquid Chromatography

In recent years, a face-lifting process has occurred in many phases of liquid chromatography. This has been due to (1) the advancement of theory, (2) the design and development of high sensitivity and low volume detectors, (3) apparatus suitable for operation at elevated pressures, and (4) advances in the development of column packing materials. During the past year and a half, extensive efforts have been exerted in our

laboratory in striving for control of the extremely important (but not always readily applied) discipline of high performance liquid chromatography. The following sections will discuss the progress of these efforts.

1. Liquid Chromatography Design. In last year's report [1], criteria were cited for a desirable design of a liquid chromatography system. Some components of the design may vary, depending upon the requirements of the problem at hand. Attention was given to the optimization of liquid chromatographic systems for steric exclusion (gel permeation) chromatography. Such a design in use in our laboratories may be seen in Figure 20. While the system was assembled for steric exclusion chromatography, it has versatility such that the parameters of

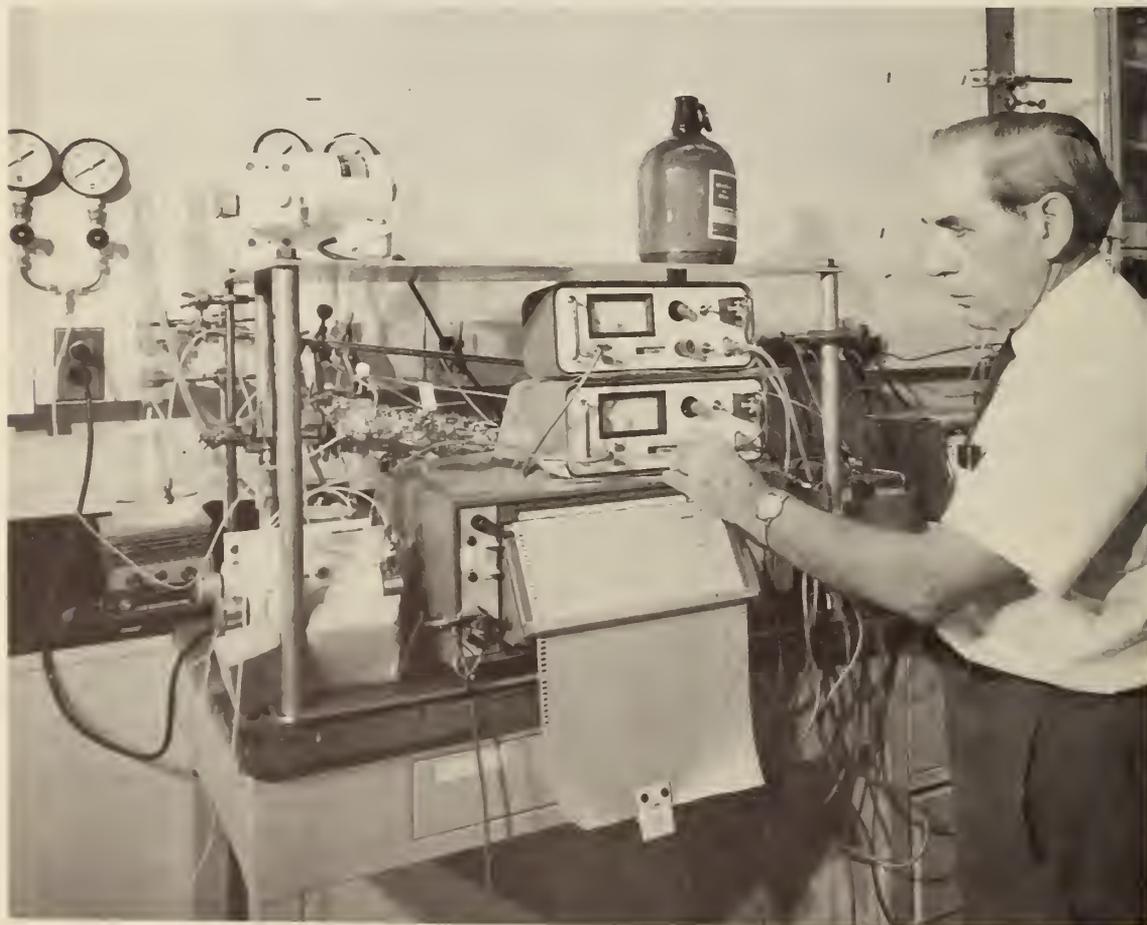


Figure 20. Steric exclusion chromatographic system.

column length and packing, temperature, flow rate, and type of detection are easily changed. Certain modifications, such as low dead volume teflon column end fittings, on-column injection, pump pulsation dampening, and reference stream recycling were investigated and incorporated into the SEC system. Chromatograms illustrating the performance of the system are given in other sections of this report.

In the past several months, investigation has begun in liquid-solid chromatography, using silica gel as the stationary phase, and a gradient mobile phase. When proper choice is made of the mobile gradient profile and its composition, diverse separations are possible for this system. A picture of the chromatographic system for silica gel chromatography is given in Figure 21, showing the use of the moving wire flame ioniza-



Figure 21. Liquid-solid chromatographic system.

tion detector. A simple gradient generator is shown in Figure 22. A composition-time profile of an n-hexane:chloroform gradient may be seen in Figure 23. The data for this curve was obtained by withdrawing aliquots from a side-arm on the pump outlet with a syringe and analyzing these by gas-liquid chromatography (GLC) using a Ucon Oil capillary column. A GLC chromatogram of one of the data points is given in Figure 24. Increasing the volume of the mixing chamber or using diminished chloroform:hexane ratios in the solvent reservoir upstream of the mixing chamber will result in a lower gradient slope. Although the usual use of mobile phase gradients is from less polar to more polar phases, the choice and manipulation of

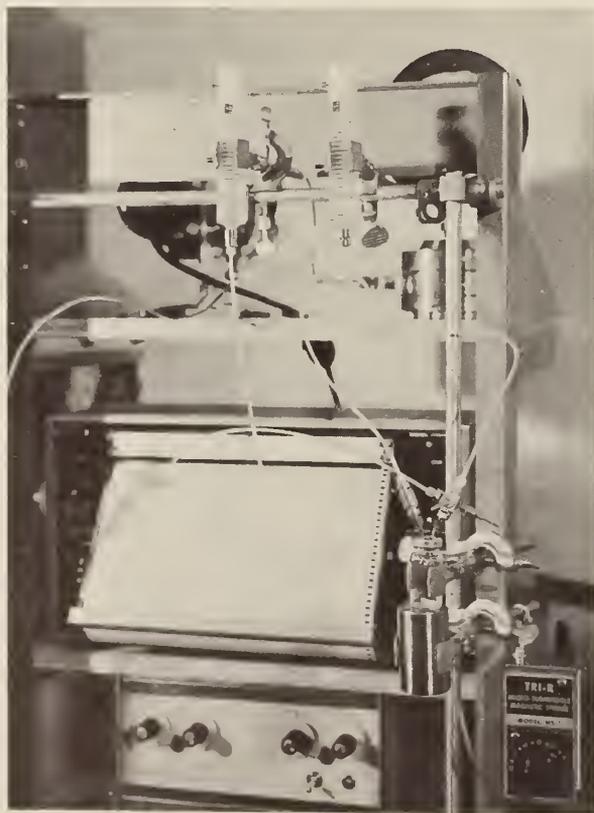


Figure 22. Proportional gradient generator.

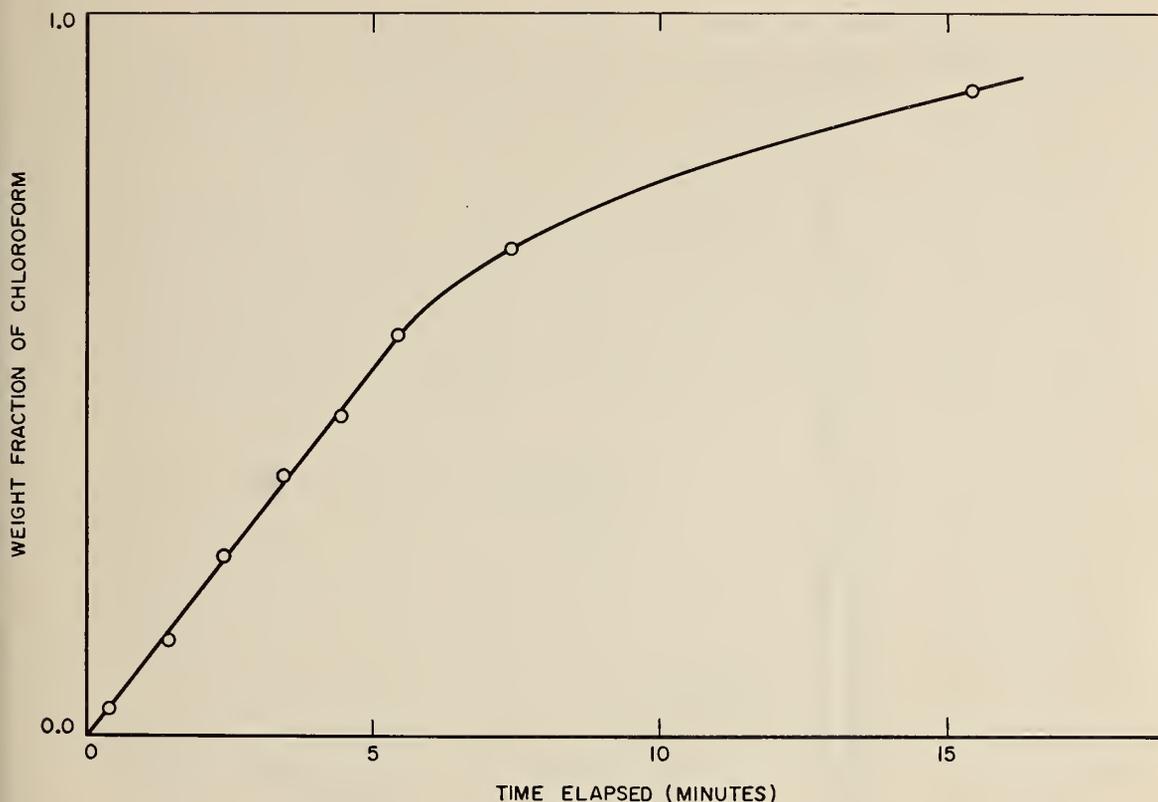


Figure 23. Gradient profile of n-hexane:chloroform.

solvents is strictly at the discretion of the experimenter. Moderated mobile phases (i.e., the addition of 1 percent or less of a polar solvent to a non-polar mobile phase) after the work of Maggs [7], Scott, and others is likewise under investigation.

## 2. Steric Exclusion Chromatography Selectivity.

The equations for resolution received development by Giddings [8] and were applied to steric exclusion chromatography [9]. A chromatographic system may be made extremely efficient and capable of eluting solutes having low band-spreading. Such a system however, is useless unless the column packing is capable of separating the solute bands from one another. Various models for selectivity are given in the excellent review article by Altgelt [10]. Much work has been reported on the separation

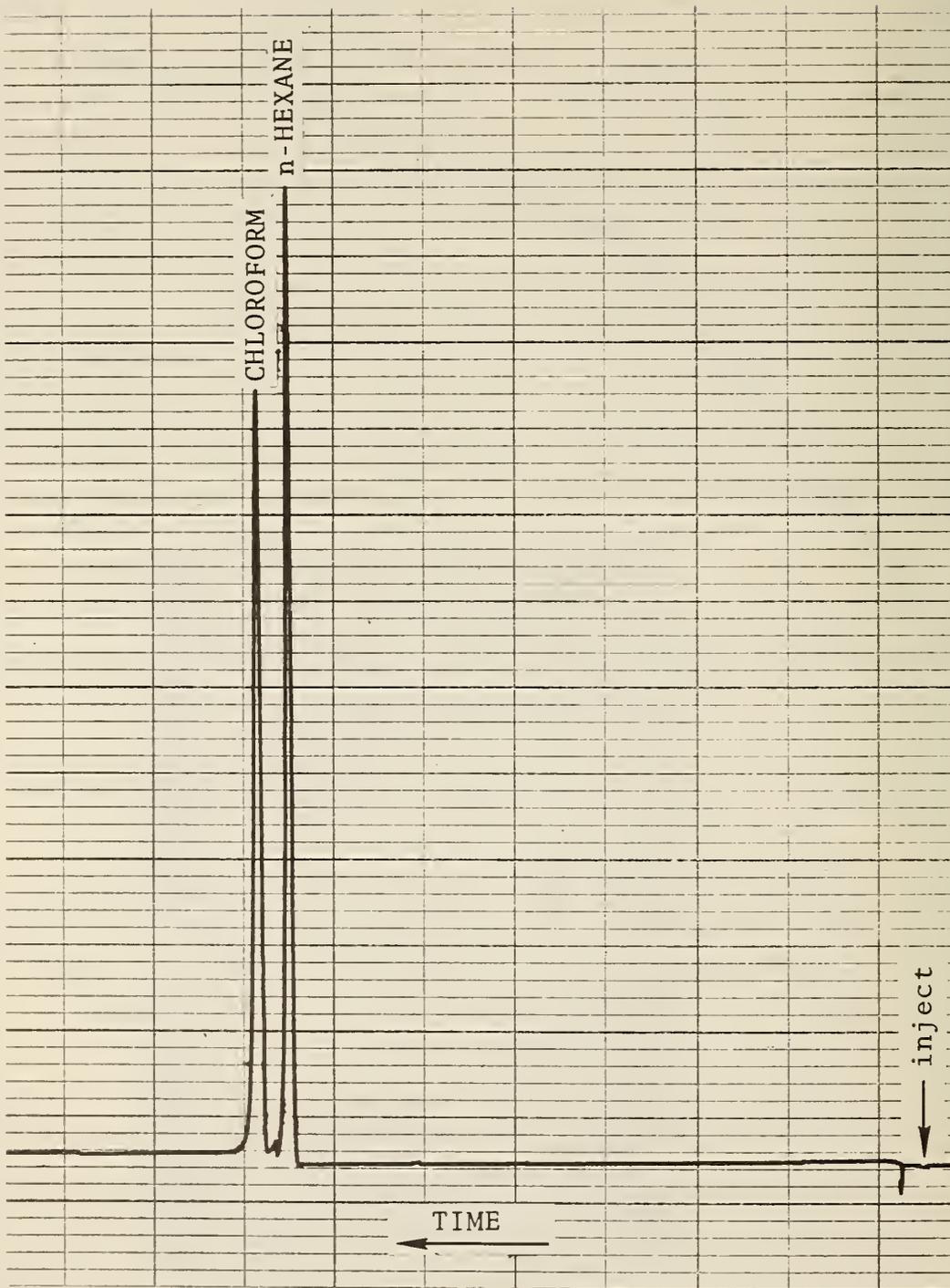


Figure 24. Gas chromatogram of mobile phase composition during gradient generation.

of high molecular weight polymers in the literature. On the other hand, our interests have principally been concerned with the separation of relatively low molecular weight solutes ( $M=700$  and less). An example of steric separation of a solute mixture of varying molecular weight is shown in Figure 25. Since the average pore diameter of the gel particle may considerably influence retention behavior, we investigated the role of DVB crosslinking in styrene/DVB copolymers and the data is given

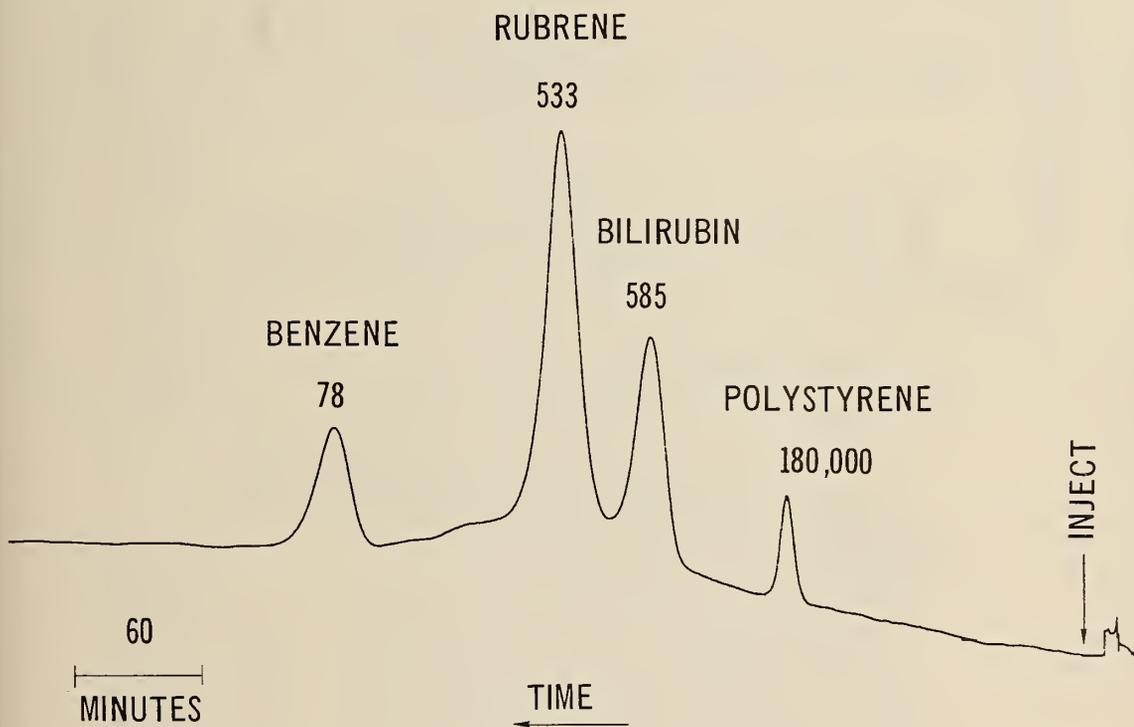


Figure 25. Steric exclusion chromatography of organic solutes.

in Table 3 and plotted in Figure 26. We have developed infrared spectrophotometric techniques for the qualitative identification and quantitative estimation of DVB content of styrene/DVB copolymers, and this is presented in another section in this report.

Table 3. Retention behavior of solutes as a function of degree of copolymer crosslinking.

| Solute                    | MW      | Copolymer crosslinking (% DVB) |               |       |             |       |             |
|---------------------------|---------|--------------------------------|---------------|-------|-------------|-------|-------------|
|                           |         | 4%                             |               | 5.5%  |             | 8%    |             |
|                           |         | $V_e^a$                        | $V_{i,acc}^b$ | $V_e$ | $V_{i,acc}$ | $V_e$ | $V_{i,acc}$ |
| Polystyrene 180,000       | 180,000 | 10.8                           | 0.0           | 9.2   | 0.0         | 10.0  | 0.0         |
| Polystyrene 4,000         | 4,000   | 10.8                           | 0.0           | 9.2   | 0.0         | ---   | ---         |
| Polystyrene 2,100         | 2,100   | 11.1                           | 0.2           | 9.3   | 0.1         | ---   | ---         |
| Polystyrene tetrastearate | 1,202   | 10.8                           | 0.0           | ---   | ---         | ---   | ---         |
| Polystyrene 900           | 900     | 11.1                           | 0.2           | 9.4   | 0.2         | 10.5  | 0.5         |
| Polystyrene 600           | 600     | ---                            | ---           | 10.8  | 1.6         | 11.2  | 1.2         |
| Rubrene                   | 533     | ---                            | ---           | 11.7  | 2.5         | 12.4  | 2.4         |
| m-Quinquephenyl           | 382     | 18.8                           | 7.9           | 13.3  | 4.1         | ---   | ---         |
| Perylene                  | 252     | ---                            | ---           | 18.5  | 9.3         | ---   | ---         |
| 3,4-Benzopyrene           | 252     | 26.1                           | 15.2          | ---   | ---         | ---   | ---         |
| Triphenyl                 | 228     | 25.6                           | 14.7          | 17.7  | 8.5         | ---   | ---         |
| Naphthacene               | 228     | ---                            | ---           | 18.2  | 9.0         | 14.0  | 4.0         |
| Phenanthrene              | 186     | 25.7                           | 14.8          | 17.7  | 8.5         | ---   | ---         |
| Anthracene                | 178     | ---                            | ---           | ---   | ---         | 17.0  | 7.0         |
| Benzene                   | 78      | 25.7                           | 14.8          | 18.0  | 8.8         | 17.3  | 7.3         |
| Nitromethane              | 61      | 26.2                           | 15.3          | 19.7  | 10.5        | ---   | ---         |

$V_e^a$ : Elution volume in  $cm^3$ .

$V_{i,acc}^b$ : Accessible gel volume; the retention volume due to solute permeation.  
 $V_{i,acc} = V_e$  (permeating solute) -  $V_e$  (excluded solute).

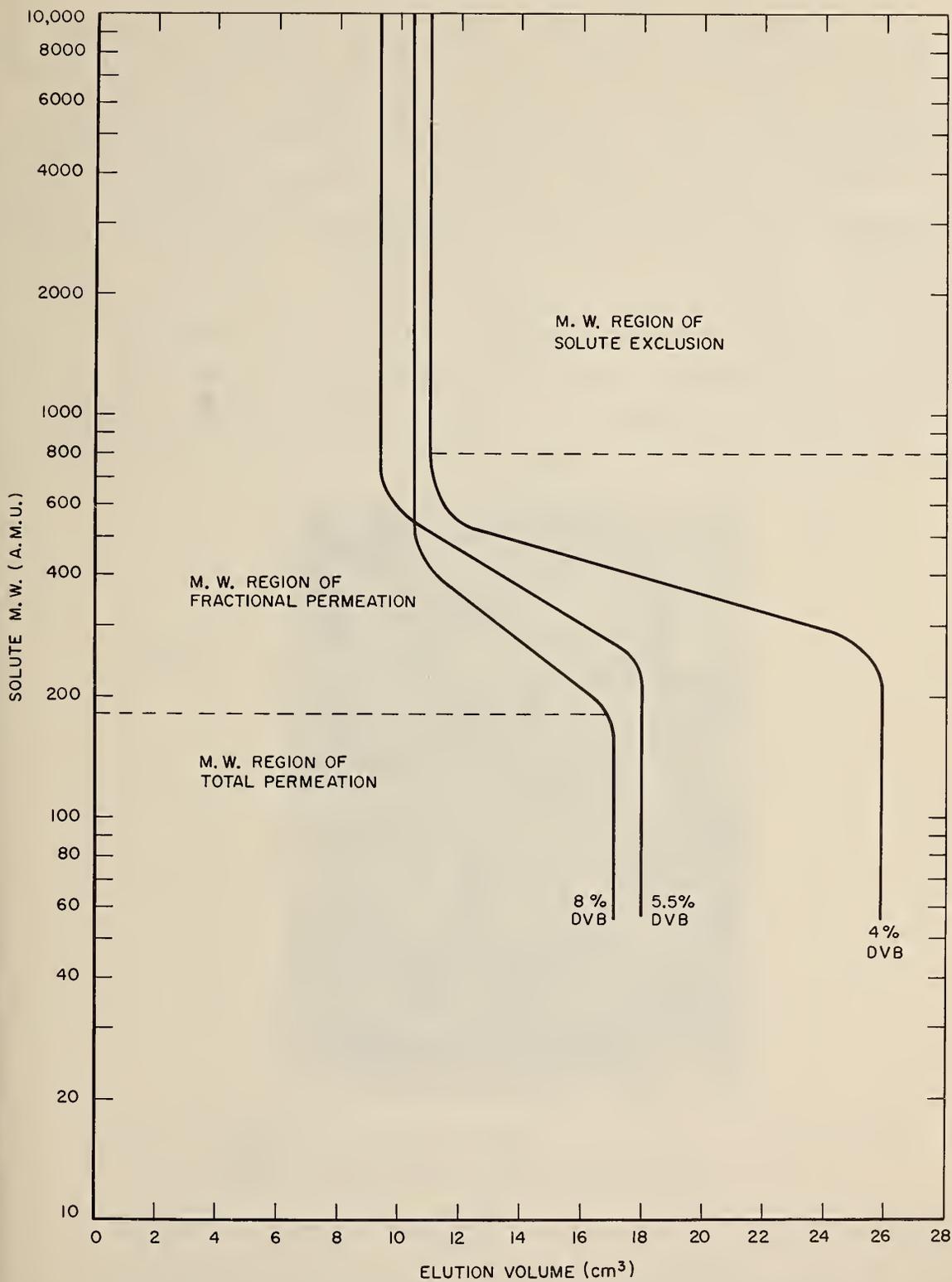


Figure 26. Retention as a function of degree of copolymer crosslinking.

It is perhaps unfortunate that this technique lacks adequate selectivity in the separation of solutes of close molecular weight (actually, molecular volume). We have initiated what we feel could become an important development in the use of gels for small organic molecule separations. Principally, this involves the synthesis of styrene/DVB copolymers containing chemical selectivity. The first such derivative prepared was a nitro derivative in which nitration occurred throughout the copolymer matrix. An example of nitrated beads evidencing strain character is shown under crossed-polars in Figure 27. Ayres and Mann earlier showed that nitrated copolymers may be useful in the separation of

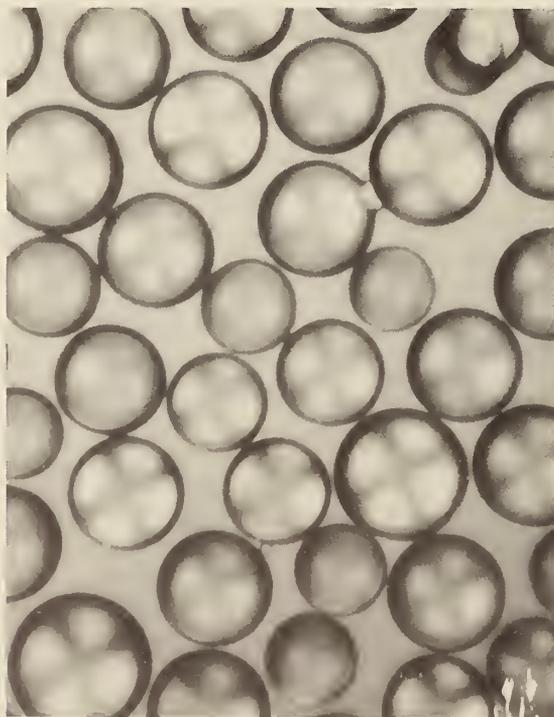


Figure 27. Photomicrograph of nitrated poly(styrene/DVB) beads under crossed-polars.

polyaromatic hydrocarbons [11] via  $\pi$ - $\pi^*$  charge transfer complexation. The use of chemically modified copolymers offering separations based on both solute size and dipole interactions are the non-ionic analog of ion exchange beads.

The development of such copolymers containing other kinds of functionality is of interest.

Attention was given to the evaluation of band spreading in steric exclusion chromatography. Employing small diameter beads (10-30  $\mu\text{m}$ ), the effect of flow rate on solute band spreading ( $H$ ) was investigated. Figure 28 shows such a plot

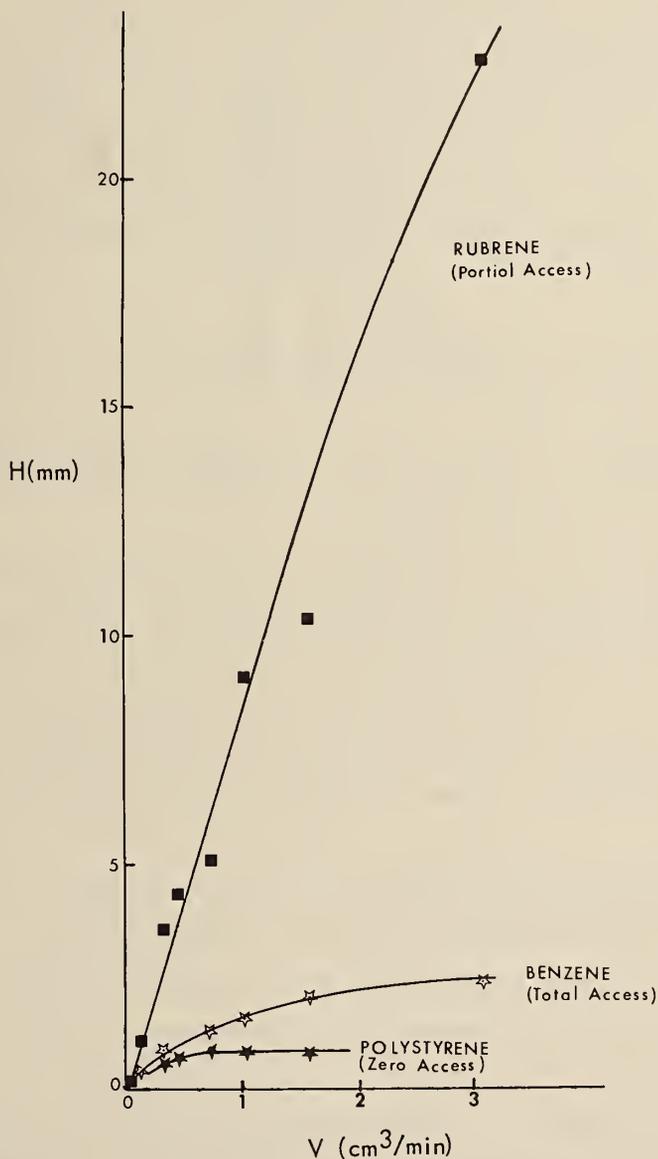


Figure 28. Plate height vs. flow rate for solutes having zero, partial, and total access to the gel interior in SEC.

for an excluded solute (polystyrene 180,000 MW), a solute that is free to permeate all pores of the gel (benzene) and is non-selectively retained, and solute of intermediate size which permeates only a fraction of the pores (rubrene). In the first case (PS180K), the internal volume of the gel phase ( $V_i$ ) is unaccessible and thus  $V_{i, \text{ accessible}} = 0$ . In the last case (benzene),  $V_{i, \text{ accessible}} \cong V_i$ . For rubrene, however,  $0 < V_{i, \text{ accessible}} < V_i$ , and it is here that flow rate dependence is important, as can be seen from Figure 28. Low plate heights are still achievable, but only at low flow rates and at the cost of analysis time. This can readily be understood from the plate height equation for steric exclusion chromatography [9]

$$H = \left[ 4D_m / 3Rv \right] + \left[ R(1-R)d_p^2 v / 20D_m \right] + \Sigma \left[ 1/0.5 \lambda_1 d_p + D_m / \omega_i d_p^2 v \right]$$

where the factor  $R(1-R)$  in the nonequilibrium term approaches its maximum value of 0.25 for solutes where  $0 < V_{i, \text{ accessible}} < V_i$ , and where  $H$  as a function of  $R(1-R)$  increases with increased flow rate ( $R$  = the ratio of solute velocity to mobile phase velocity). At high mobile phase flow rates,  $v$  in the nonequilibrium term (middle term) contributes to increased values of  $H$ .

3. Liquid Chromatography Interactions. Liquid chromatography using a 2 mm I.D. stainless steel column packed with OPN (oxypropionitrile) "brush" particles [12] gave indications that serious tailing problems can arise for carbonyl-containing solutes. In situ silylation of the column packing did not improve matters, and indeed, caused at least partial removal of the "brushes" as indicated by the appearance of a yellow colloid in the column effluent. A separation of a solute mixture is given in Figure 29. This was only obtained at low mobile phase velocities and with the use of a solvent moderator

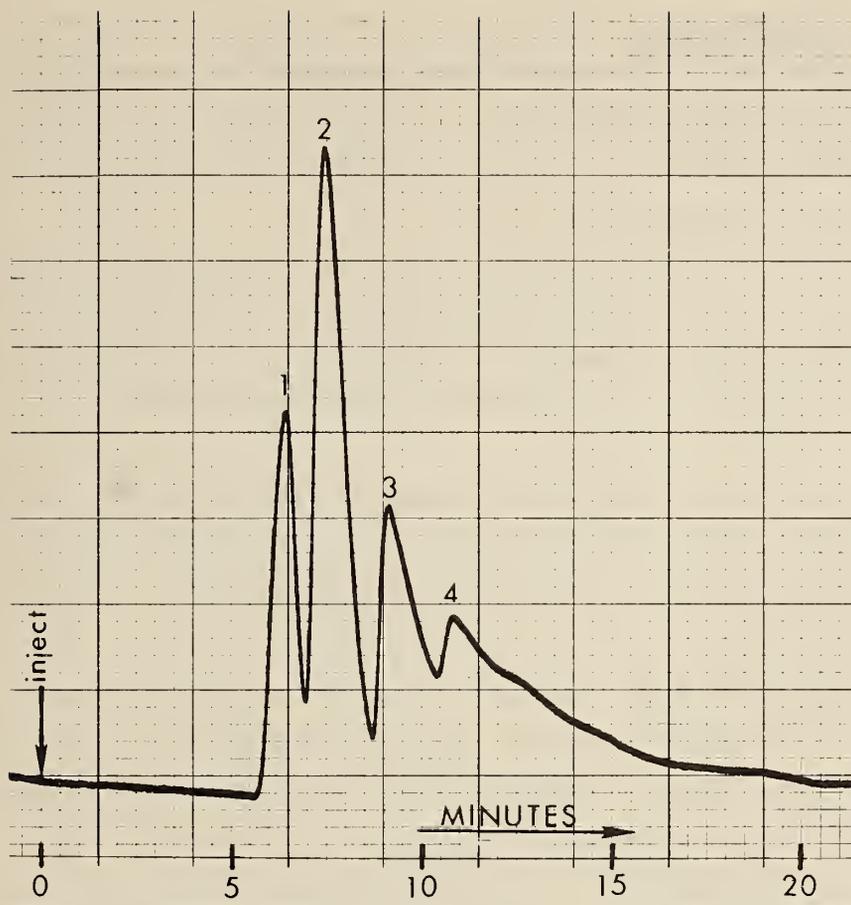


Figure 29. Separation of a multifunctional solute mixture on oxypropionitrile.

of 0.5 percent of methyl ethyl ketone. The relatively more rapid separations of solute mixtures containing only one type of functionality is reported on chemically modified pellicular (solid core) particles [13] in which the diffusion phenomena associated with porous silica particles is negligible and rapid solute equilibrium between mobile and stationary phases occurs. The capacity, however, of pellicular particles for solute sample size is small.

4. Detector Evaluation. In the past year, we have employed three different detectors: (a) differential UV absorption at 254 nm, (b) differential refractive index (RI) detection in organic and aqueous mobile phases, and (c) pyrolysis-flame ionization detection using the moving wire detector.

a. Advantages. The UV detector was the most sensitive detector and proved to be extremely satisfactory for steric exclusion chromatography of bilirubin and standard aromatic solutes. Detection of  $10^{-7}$  g of bilirubin is readily accomplished with this detector.

The RI detector has sensitivity limits that vary with the magnitude of the RI difference between solute and solvent. The RI detector tends to have the advantage of being less selective than the UV detector. We could detect  $10^{-5}$  g of bilirubin.

Both the RI and UV detectors which we have utilized have low volume flow cells in the order of several microliters, relatively fast response, and high sensitivity for their relative functions.

The pyrolysis flame ionization detector has a clear potential for becoming the most useful detector for the liquid chromatography of organic solutes. It is well suited for gradient elution techniques.

b. Disadvantages. Both the RI and UV detectors which we have employed in our investigations are somewhat sensitive to effluent flow rate changes. The former is the most affected and is difficult to use with flow and temperature-programming, and mobile phase gradients. The moving wire flame ionization detector performs in an excellent manner for each of these techniques. Its major difficulties however, lie in poor efficiency of transfer of the effluent mobile phase to the wire (presently only about 2 percent), and in variations in the pyrolysis efficiency relative to the solutes in question (although a solution to this seems to have been provided [14]).

5. Interactive Copolymer Gels. The theory of restricted diffusion in SEC, while a reasonable concept--and there are indications by workers using controlled pore glass particles [15] that such a phenomenon can exist--predicts the pronounced tailing of solutes and a dependence of solute elution volumes

( $V_e$ ) on flow rate. Minimal or no tailing is usually observed in SEC, however, excluding adsorption, sample overloading, or other extraneous effects [16]. The separations phenomena on styrene-DVB copolymers can primarily be explained on the basis of steric exclusion. It has been observed that the selectivity of copolymer gels for molecular size separations of chromatographed solutes is often adequate for polymer mixtures, but extremely poor for small organic molecules where solutes having only small molecular weight differences have identical retention volumes.

The introduction of specific functional groups to the copolymer matrix may broaden the basis for selectivity using copolymer gels. Being non-ionic, their capability of swelling in organic solvents will be maintained and an interactive gel system can be attained. Such non-ionic modification of silicious particles has resulted in the commercial "brush" packings. The chemically-modified copolymer gels, however, avoid the hydrolysis problems associated with silicious "brush" particles and provide microscopic three-dimensional interaction for solute molecules entering the gel phase. The first derivative prepared was a nitrated styrene-DVB copolymer in which nitro groups are attached to the benzene rings of styrene. Optical examination of this derivative preparation under crossed polars may be seen in Figure 27. The implication here of the application of copolymer gels containing polar groups which interact predominantly with permeating solutes offers a separations potential not possible with the unmodified styrene-DVB gels conventionally used in SEC. Experiments will broaden in the coming year in both derivative synthesis and chromatography to ascertain the extent of this implication.

### C. Standard Reference Materials

#### 1. Clinical

a. Bilirubin. In last year's report [1] we indicated some of the preliminary difficulties associated with bilirubin

analysis using liquid chromatography. In the past year, extensive work has occurred on this problem and substantial progress has been made. The system ultimately chosen and used throughout this work has been steric exclusion (gel permeation) chromatography employing chloroform as the mobile phase and styrene-DVB copolymer beads ( $d_p = 10-30 \mu\text{m}$ ) as the column packing.

(1) Packing the Column. The column may be packed with preswollen beads in a chloroform slurry. However, difficulty may be encountered when an upright column is packed from the top, due to the solvent density being greater than the swollen gel particles. This results in a portion of the packing migrating to the top of the column even under pressure, and the situation becomes increasingly difficult with low crosslinked copolymers. This unfavorable situation was resolved by pre-swelling the beads in a solvent mixture (benzene:chloroform of 2.38:1, v/v) having the same density as the copolymer ( $\rho = 1.06$ ), and packing the column from the bottom under pressure as illustrated in Figure 30. Using this technique, the column is rapidly filled in several minutes. The column is then inverted, and packing is completed from the top in the conventional manner. The column is then thoroughly purged with chloroform to remove all traces of benzene as indicated by following the UV absorption of the effluent stream. This procedure results in a savings of four-six hours over the conventional method.

(2) Operational Modifications

(a) Column End-Fittings -- Our system initially employed plastic end fittings which swelled excessively in chloroform and other organic solvents resulting in the complete blockage of flow at the column head, driving the inlet pressure gauge out of calibration. This situation was rectified by substituting machined teflon fittings (Figure 31). These fittings have been 100 percent trouble-free for the past year in continuous operating systems.

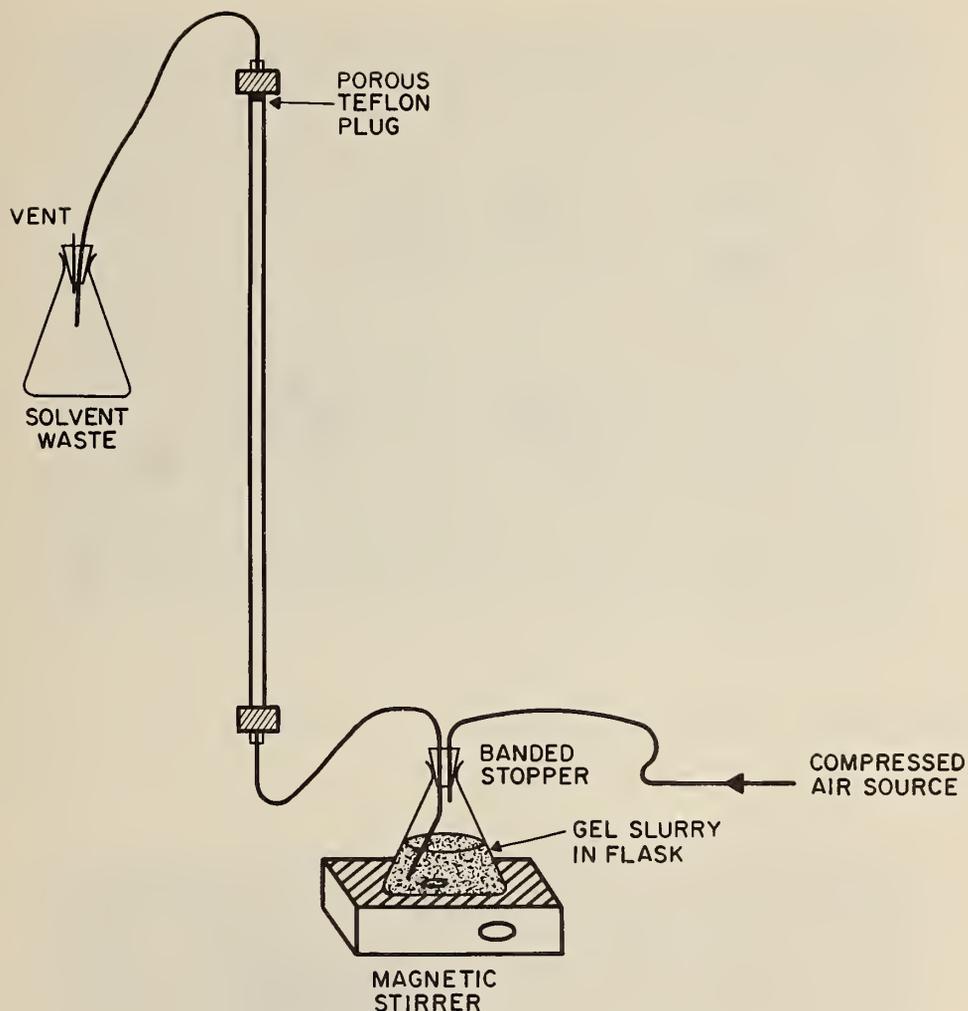


Figure 30. Packing a gel column.

(b) Sample Injection -- Although we have used both teflon switching valves with sample loops and stopped flow for sample introduction, simple syringe injection through a septum has been used almost exclusively. This has particularly been the case for SEC in which low inlet pressures (50-100 psi) are employed. A low volume injection port containing a silicone septum which is appropriate for sample injection directly onto the column bed with the pump operating, is shown in Figure 32. Sample sizes of 0.5 to 500  $\mu$ l are readily used in this manner.

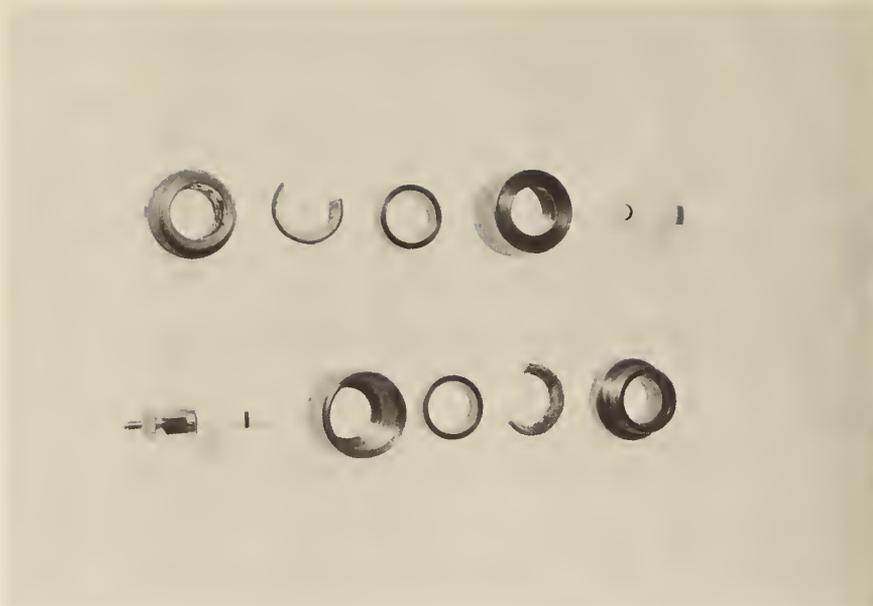


Figure 31. Column teflon end fittings.

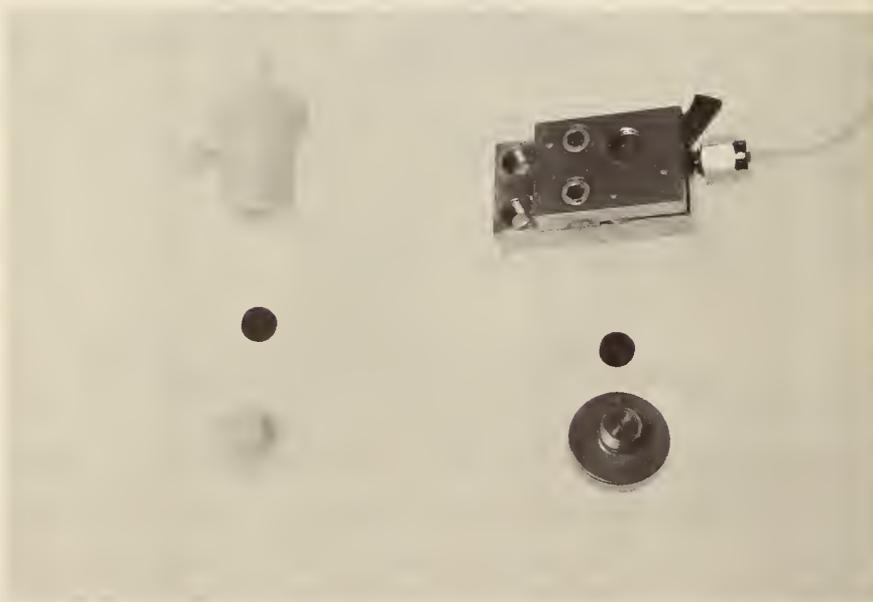


Figure 32. Sample injection port.

(c) Pulsation -- Mobile phase surges produced by pulsating pumps severely affect the signal output from low-volume, flow-sensitive detectors, unless provision for pulse dampening is introduced into the system. On the sample side of the detector, the column may or may not provide sufficient dampening action of solvent pulsing. On the reference side, pulsation in the detector is dramatic. We tried to remedy this by the use of a stainless steel capillary coil; however, this was prone to blockage at its inlet. A better solution is the use of a three meter length of thin-wall teflon tubing (0.125 inch o.d. by 1.5 mm i.d.) which expands slightly with pulsation, followed by a 5 cm stainless steel tube packed with 30/60 mesh silica gel, both located upstream of the column. The steel-teflon couple acts as a capacitive device which results in lowering the gauge fluctuations from 13 psi to less than 2 psi at 200 psi inlet pressure. The reference stream is adequately dampened by a column of 30-60  $\mu\text{m}$  silica gel, shown in Figure 33.

(d) Reference Stream Recycling -- The consumption of mobile phase is avoided by return of the reference stream effluent from the detector to the solvent reservoir. This mode of operation permits overnight analysis as well as assisting full time operation of the chromatograph.

### (3) Precautions

(a) Swelling variations of a gel packing can cause rupture of a glass column having a wall thickness as great as 3 mm. Manipulation of mobile phase polarity should be carried out in stainless steel columns. In the interest of good chromatography, no mobile phase change should be introduced which severely changes the bed dimensions.

(b) Flammable or toxic mobile phases (eg. tetrahydrofuran) should be used with care, with adequate provisions for ventilation, although no problem is encountered with adequate precautions taken.



Figure 33. Pulsation dampening of the reference stream.

#### (4) Bilirubin Chromatography

##### (a) Steric Exclusion Chromatography --

Chloroform has been found to be the only suitable mobile phase. It affords sufficient solubility for bilirubin (ca.  $0.4 \text{ mg/cm}^3$ ). It is a non-reactive solvent for this compound. It is transparent in the UV at 254 nm (the wavelength used by the detector). It is also non-flammable. All analyses were performed at ambient temperature, in the dark, using an aluminum foil jacket around the column. A chromatogram of bilirubin is shown in Figure 34 on a 4 percent DVB-styrene column. Reference to

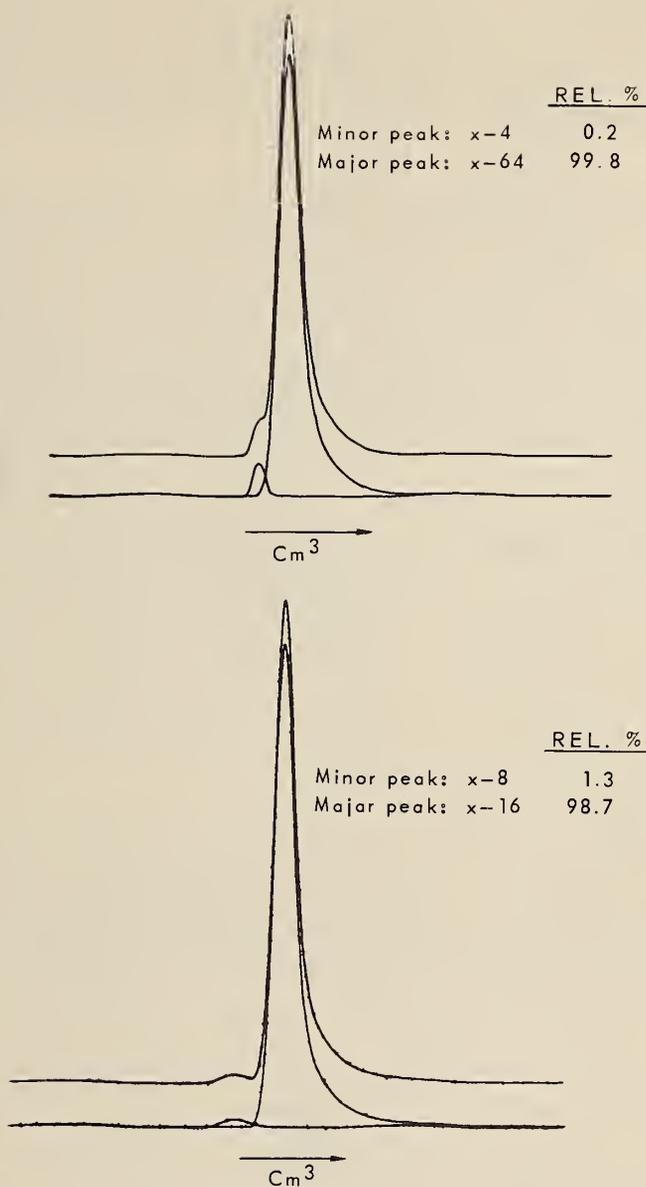


Figure 34. Steric exclusion chromatography of bilirubin.

Figure 26 shows that bilirubin (MW 585) elutes near the top of the useful portion of the standard curve. The fore-peak impurity has been consistently found in bilirubin samples, is larger in poorer grades of bilirubin, is not produced by the system, and we are sure that it is real. Its identity is not known to us and accurate measurement of its concentration in

bilirubin requires further work. Because of its position of elution, its molecular volume is slightly greater than bilirubin, and its molecular weight is in the range of  $500 < MW < 900$ .

The questions of gaussian distortion and peak integrity are difficult to answer. The conditions of low resolution chromatography have been simulated with a series of chromatograms that were calculated and plotted by a computer. The series in Figure 35 shows consecutive plots of two gaussians having a relative intensity ratio of 5 to 95 varied from  $0.5\sigma$  to  $6\sigma$  separation in increments of  $0.5\sigma$ . Two gaussians having a 10 to 90 relative intensity ratio are given in Figure 36. Finally, shown in Figure 37 is a similar series of two gaussians having a relative intensity ratio of 20 to 80. The question of peak integrity does not become suspect until at least a 1.5, 2.0, or  $2.5\sigma$  separation is achieved for a 20, 10, or 5 percent intensity contaminant, respectively.

Shown in Figure 38 is an example of another bilirubin sample which contains a lower molecular weight impurity. While its retention lies below the useful portion of the gel calibration curve, ancillary work (to be described below) substantiates that this impurity is monochlorobenzene.

(b) Gas-Liquid Chromatography -- GLC is not directly useful for bilirubin itself, owing to the high molecular weight, low vapor pressure, and susceptibility to thermal degradation of bilirubin. This technique is extremely useful, however, for the examination of volatile contaminants. Figure 39 shows a chromatogram of a bilirubin sample in chloroform. The contaminant has been identified as monochlorobenzene by mass spectrometry of the vapor from bilirubin placed in the solid probe. Its level in this bilirubin sample was estimated from GLC at 0.6 percent. A second volatile impurity was found in several bilirubin samples from GLC analysis of bilirubin in dimethylsulfoxide. This impurity, shown in Figure 40, has been

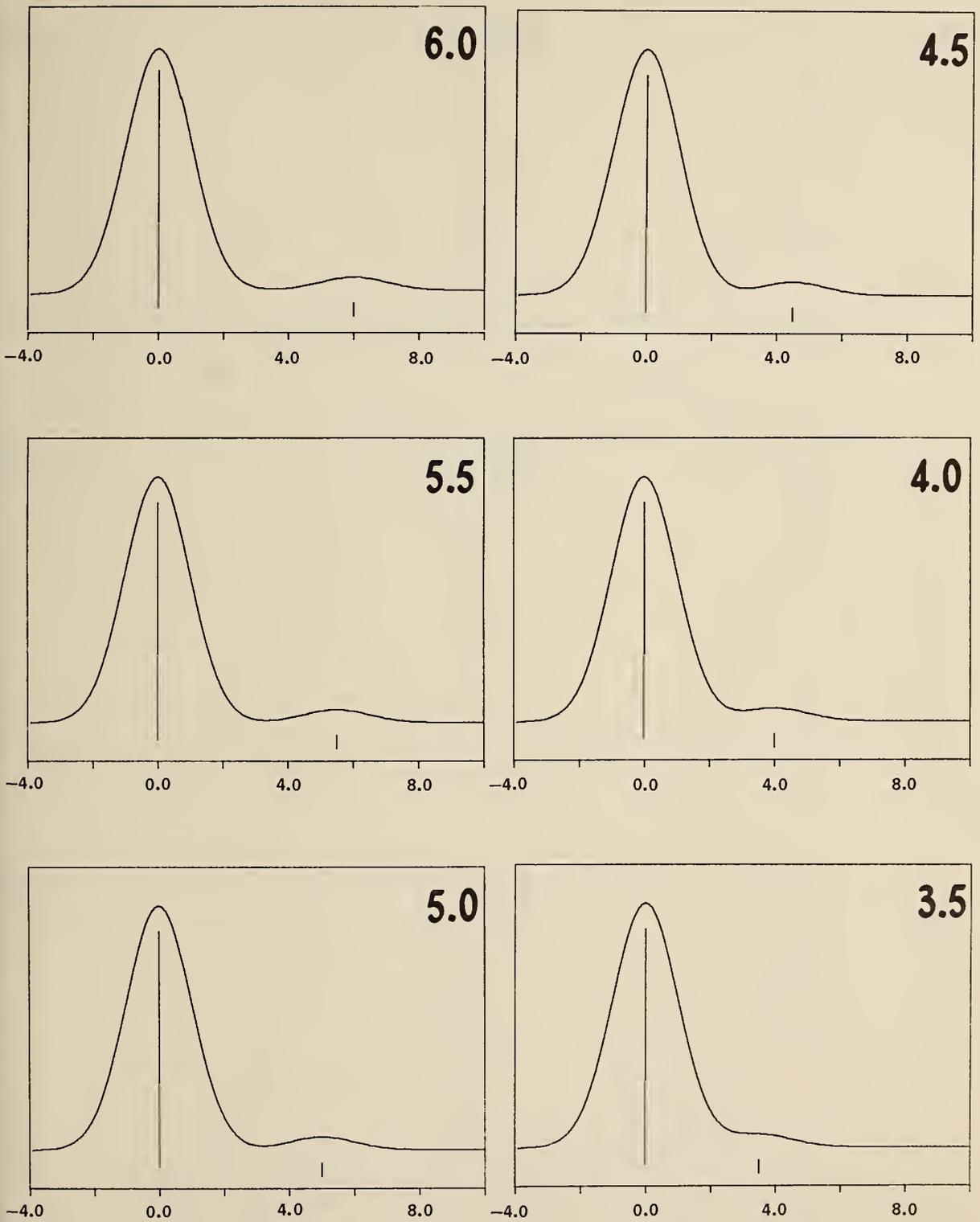


Figure 35. Separation of Gaussians with 5:95 relative intensity ratios.

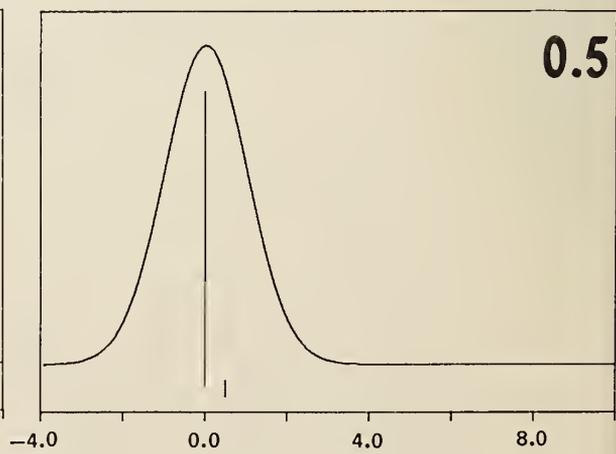
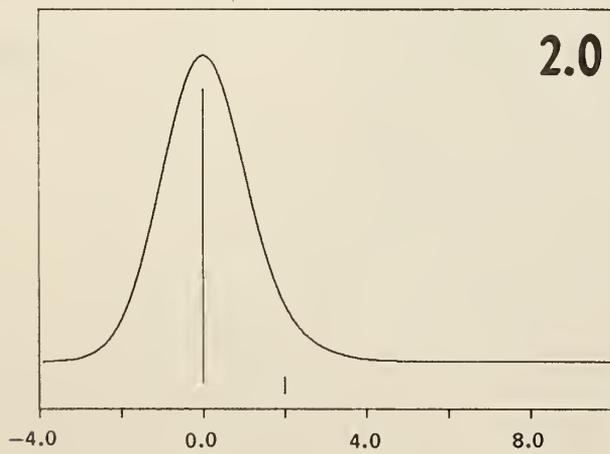
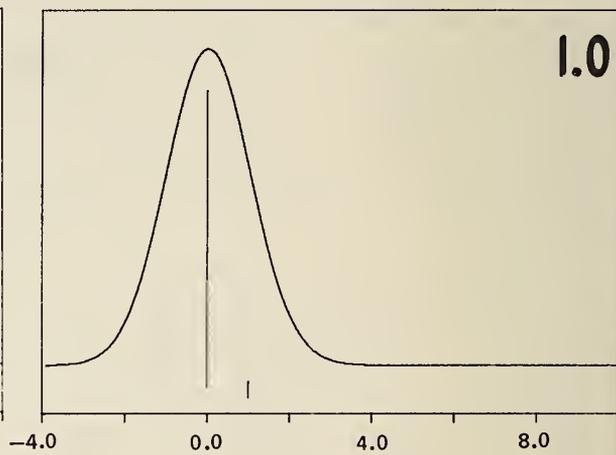
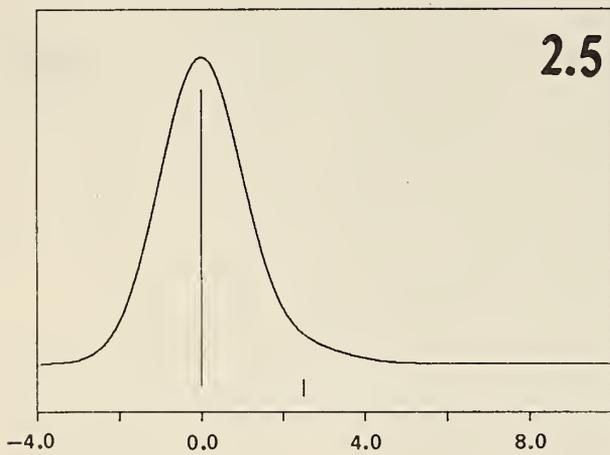
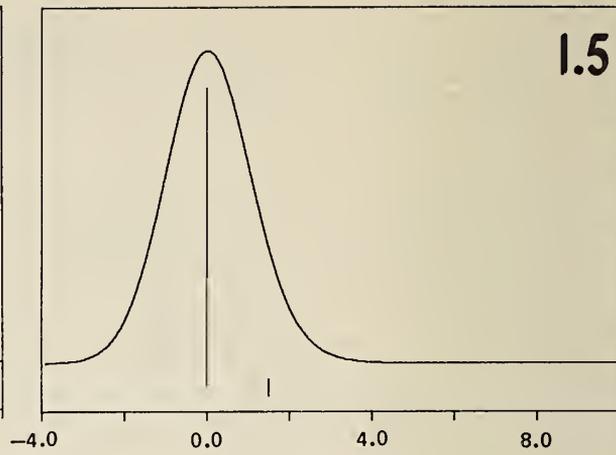
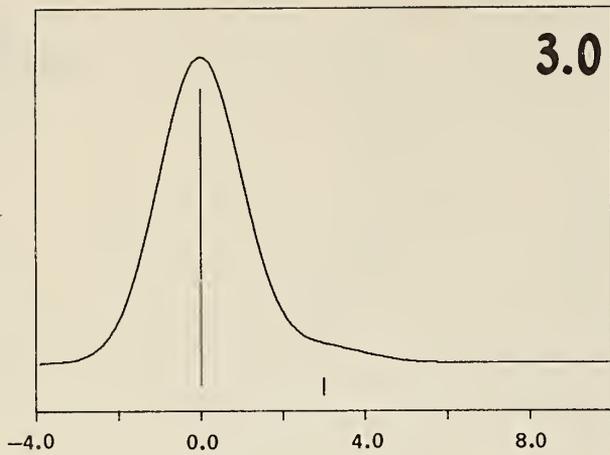


Figure 35. continued

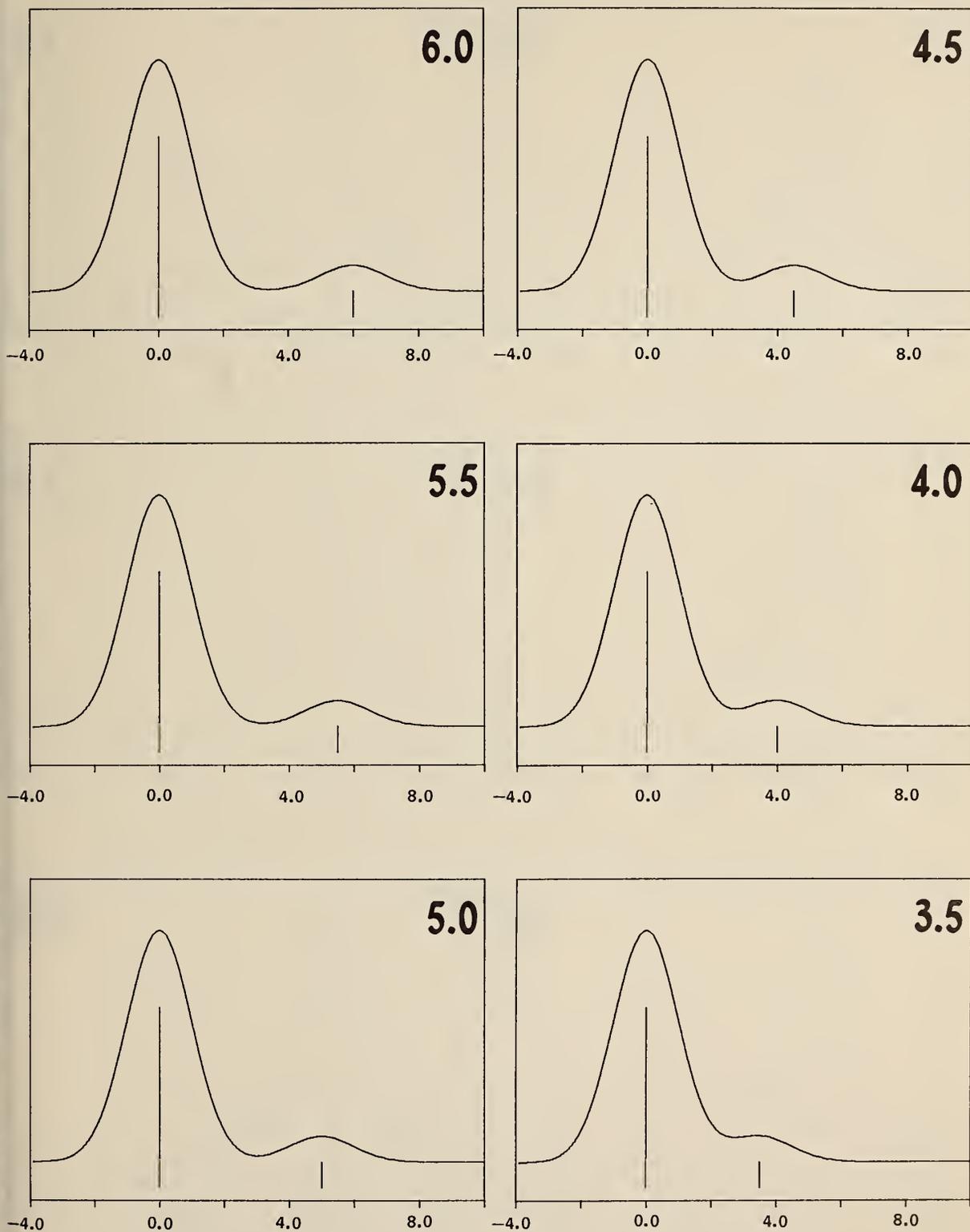


Figure 36. Separation of gaussians with 10:90 relative intensity ratios.

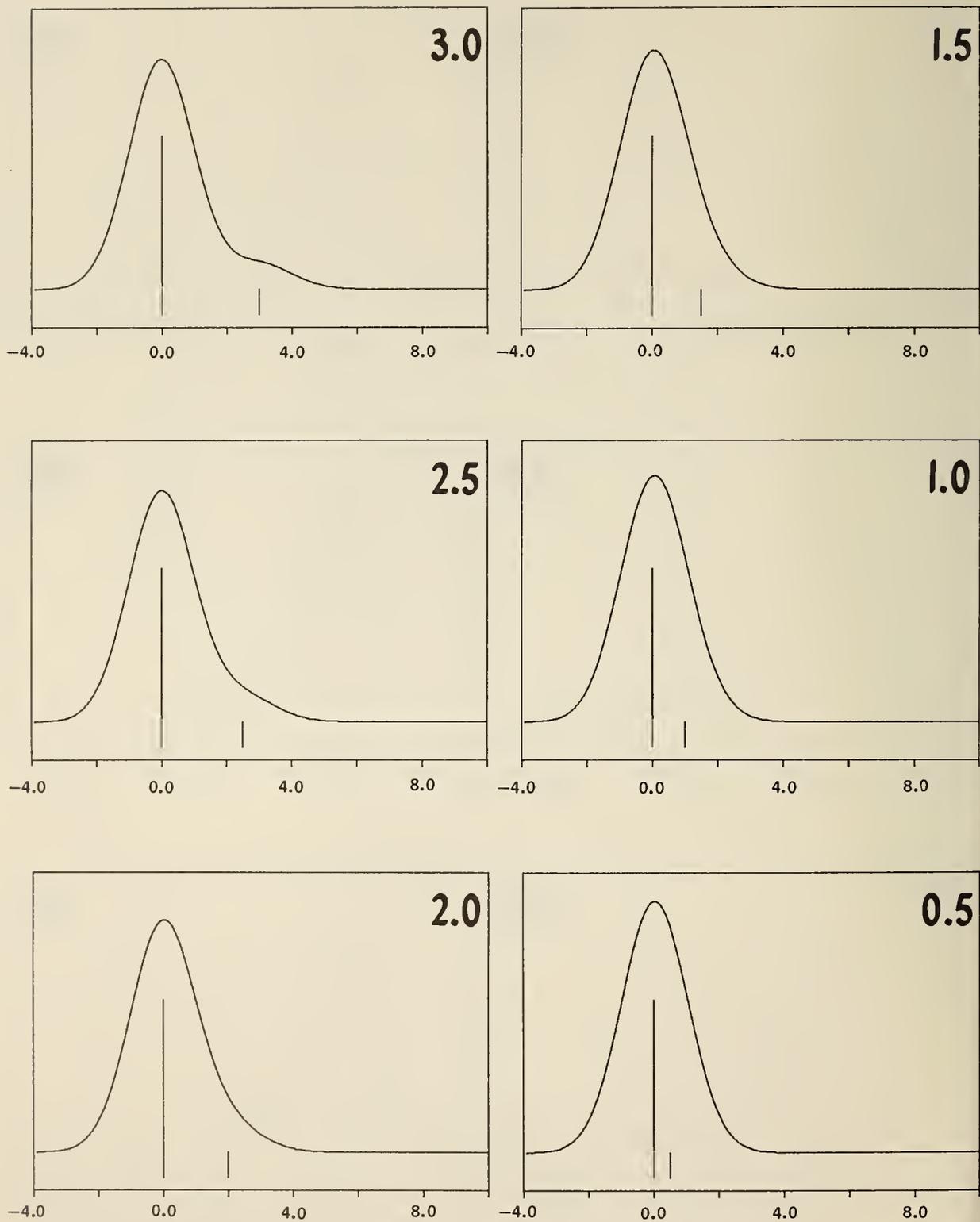


Figure 36. continued

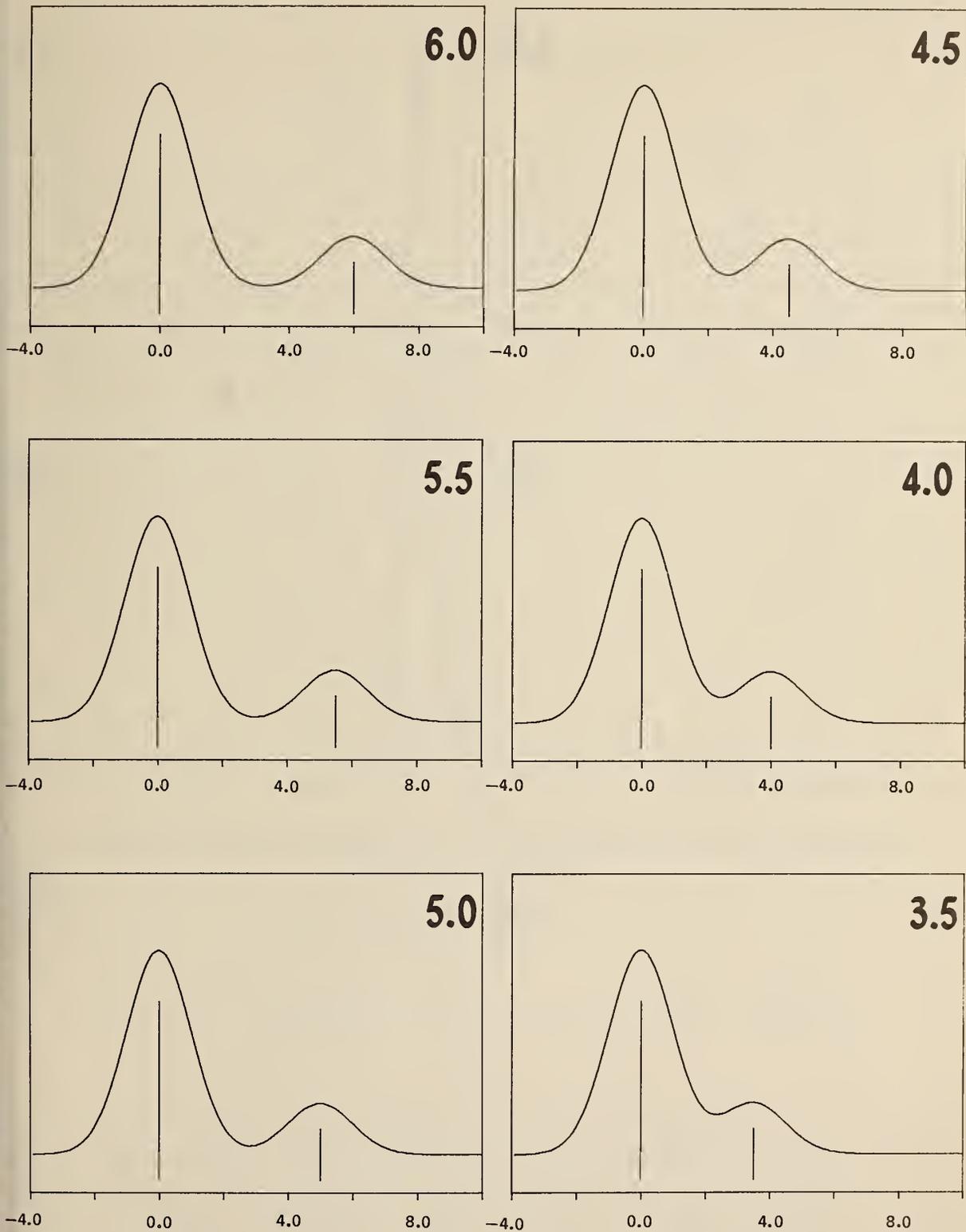


Figure 37. Separation of gaussians with 20:80 relative intensity ratios.

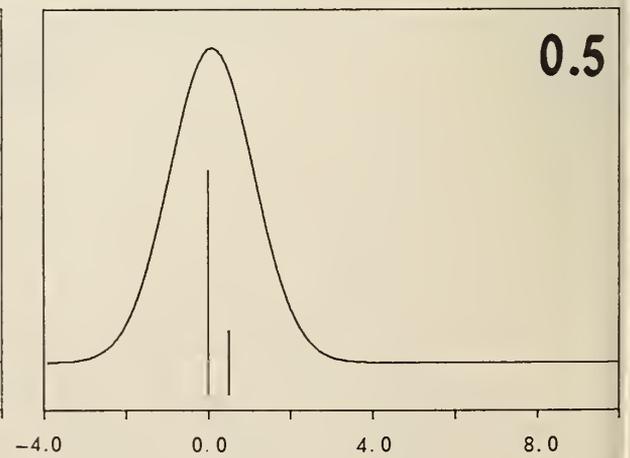
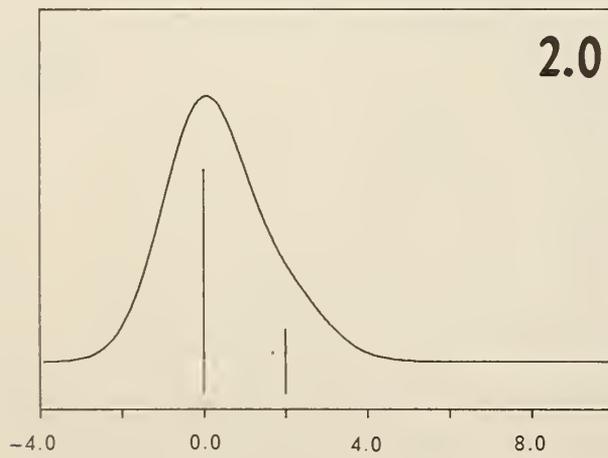
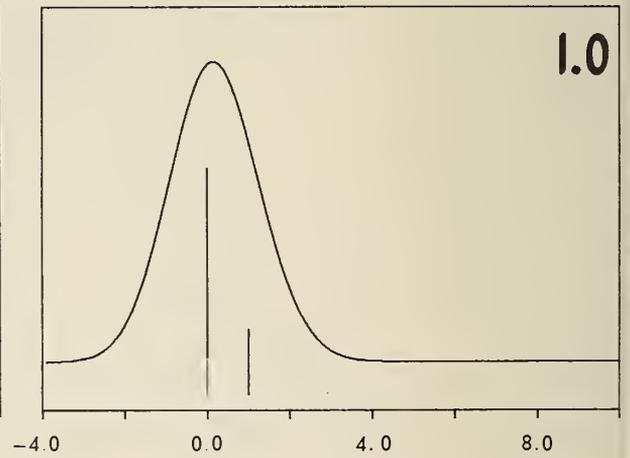
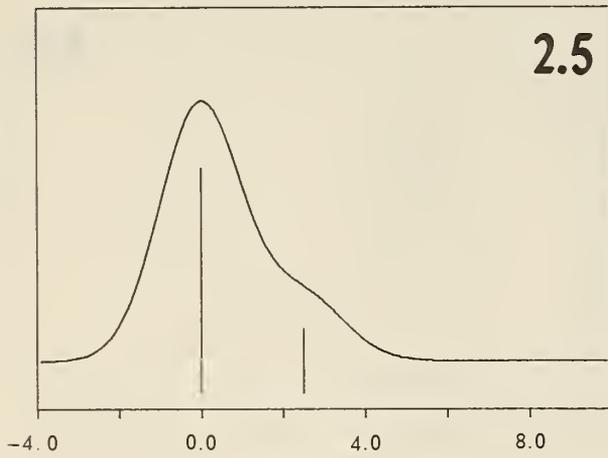
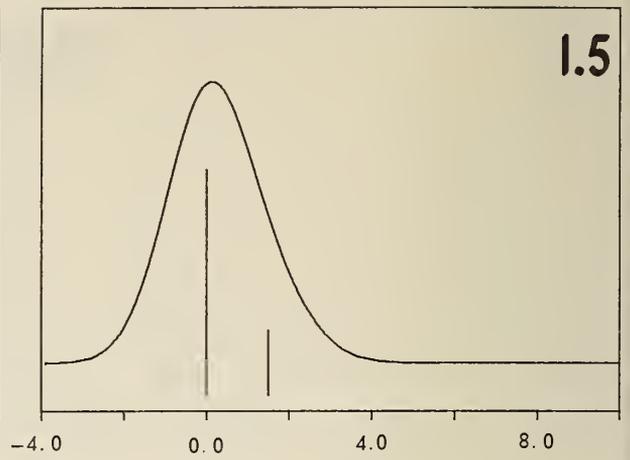
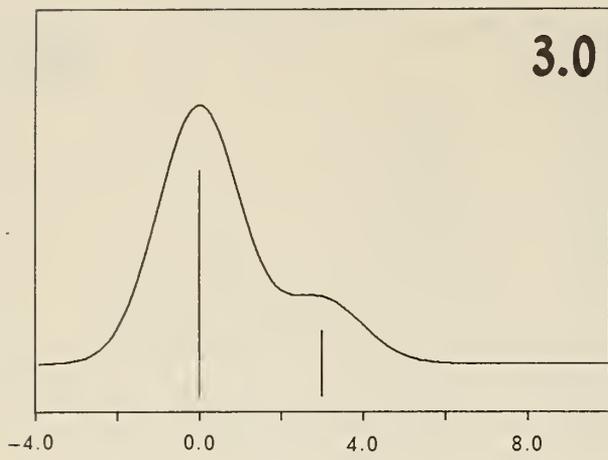


Figure 37. continued

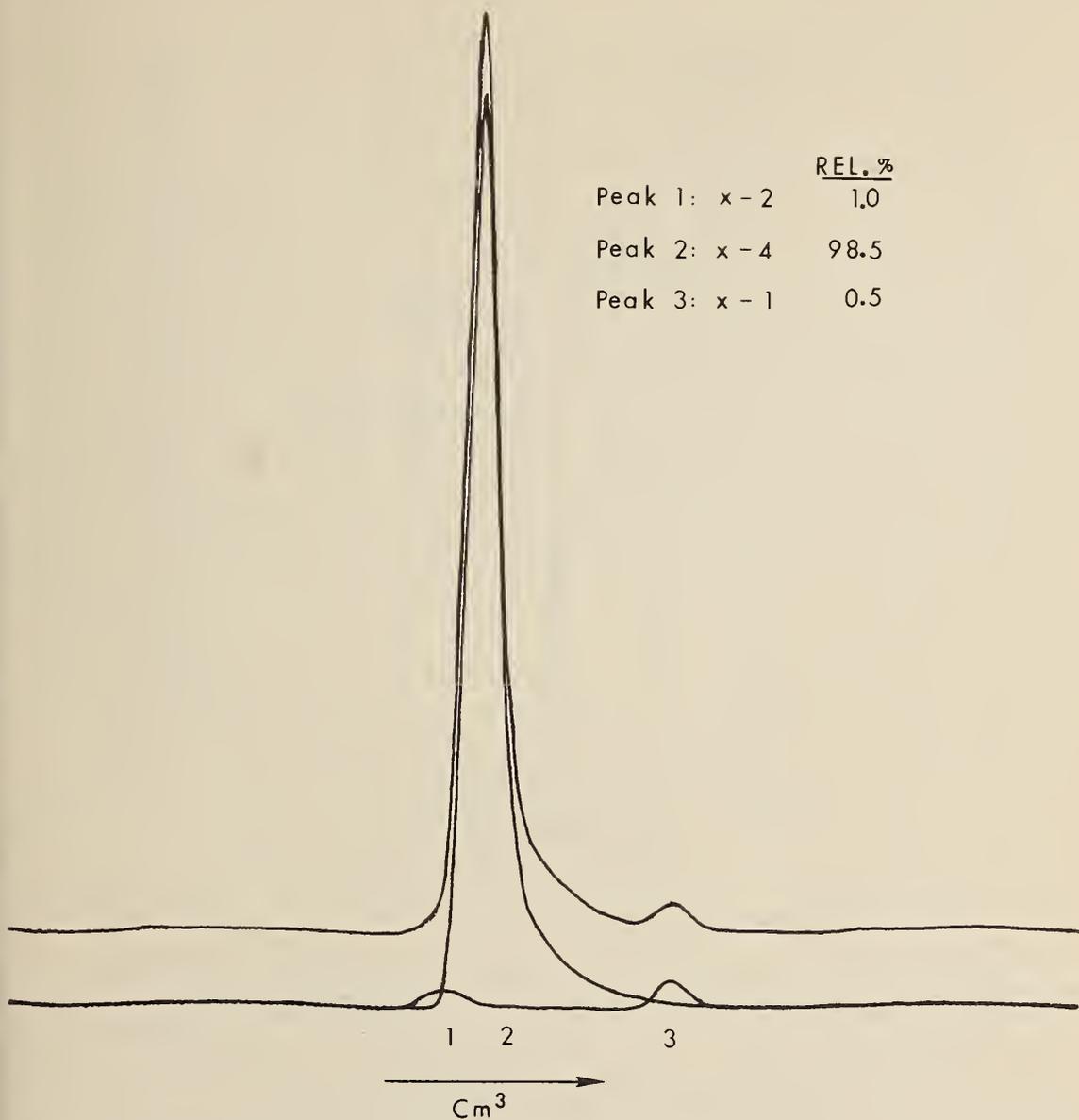


Figure 38. Detection of monochlorobenzene impurity by SEC.

identified by GLC-MS and MS solid probe techniques as chloroform. Its concentration was estimated by the standard addition technique in GLC at 1-1.5 percent. The m/e peaks observed by mass spectrometry for the identification of these two volatiles are listed in Table 4.

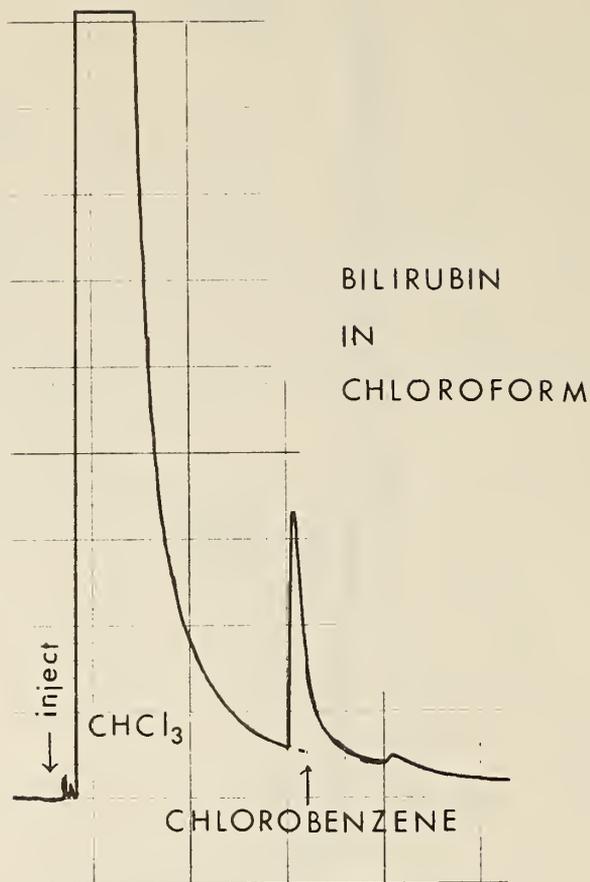


Figure 39. Detection of monochlorobenzene impurity by GLC.

Table 4. Mass spectral detection of volatile impurities in bilirubin samples.

| A. GLC/MS |                                | B. SOLID PROBE/MS |   |
|-----------|--------------------------------|-------------------|---|
| m/e       | Specie                         | m/e               | Specie  |
| 118       | CHCl <sub>3</sub> <sup>+</sup> | 112               | C <sub>6</sub> H <sub>5</sub> Cl <sup>+</sup> |
| 117       | CCl <sub>3</sub> <sup>+</sup>  | 77                | C <sub>6</sub> H <sub>5</sub> <sup>+</sup>    |
| 83        | CHCl <sub>2</sub> <sup>+</sup> | 76-72             | proton stripping                              |
| 48        | CHCl <sup>+</sup>              |                   |   |
| 47        | CCl <sup>+</sup>               |                   |   |

Volatiles: A-chloroform; B-monochlorobenzene

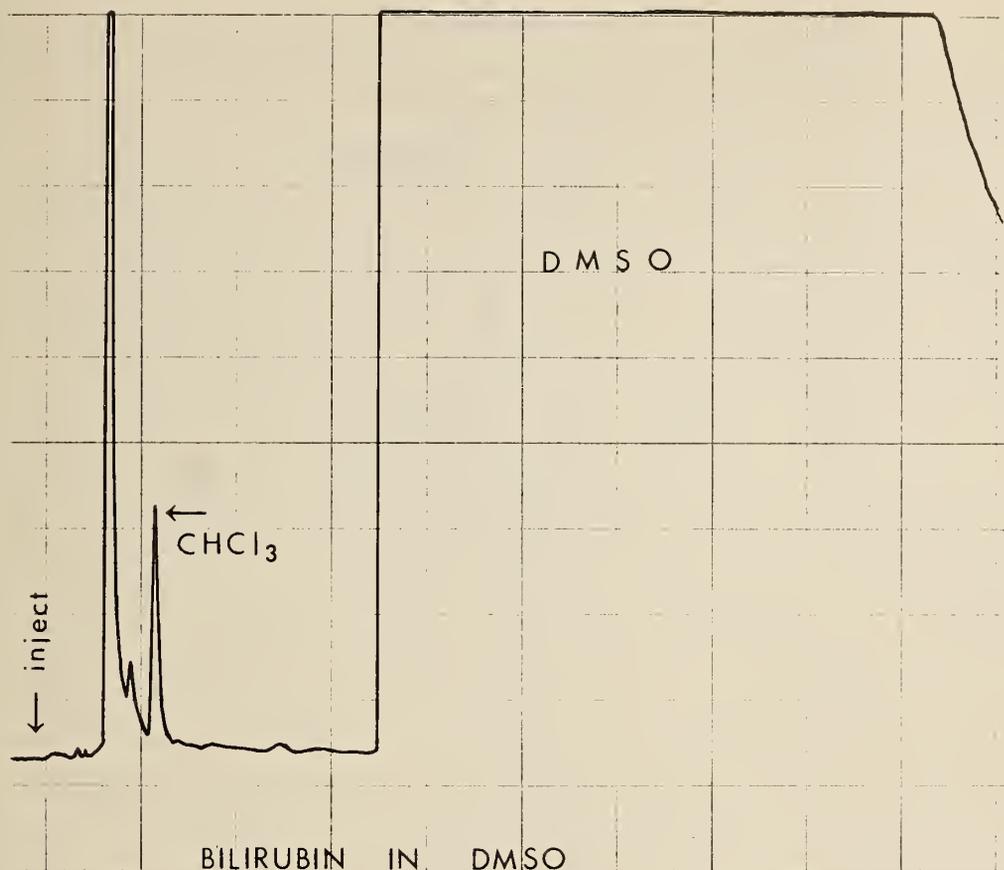


Figure 40.- Detection of chloroform impurity by GLC.

(5) Summary. Bilirubin may be readily analyzed by steric exclusion chromatography under conditions assuring its stability. Further liquid chromatographic effort is needed for complete separation and identification of its high molecular weight contaminant. The volatile impurities which may occur in bilirubin samples are monochlorobenzene and chloroform. These are not surprising since the former is utilized in the initial extraction of bilirubin from hog bile hydrolysate, and the latter is used in the final recrystallization steps. The present use of steric exclusion chromatography has afforded us a useful separations technique for a chromatographically difficult material.

b. Glucose. Investigations of glucose purity for SRM glucose were performed by ion-exchange chromatography and GLC on a sample received from Section 310.07.

Ion exchange chromatography was performed on a cation exchanger (10-30  $\mu\text{m}$  bead diameter) at 450 psi inlet pressure using degassed distilled water as the mobile phase and a differential refractometer detector. Fructose and mannose were potential impurities in glucose. The chromatograms indicated that such contamination (Figure 41) was absent at the 1 percent level. Glucose had a retention time of 20 minutes in this system. The separation factors ( $\alpha$ ) for both mannose and fructose relative to glucose were 1.10.

Gas-liquid chromatography was carried out on a 10 percent SE-30 column at a helium flow rate of 100  $\text{cm}^3/\text{min}$  and a temperature program rate of  $4^\circ/\text{min}$  from 125 to 250  $^\circ\text{C}$ . Care was taken in the silylation of glucose to produce reaction directly with the solid material rather than in a pyridine solution, to avoid anomerization. The use of pyridine in the silylation results in the conversion of 15-20 percent of the  $\alpha$  anomer to the  $\beta$  anomeric form. Separate peaks for silylated  $\alpha$ - and  $\beta$ -D glucose are observable on the chromatogram under the above conditions with a relative retention of  $\beta/\alpha$  of 1.16. Under appropriate silylation conditions, the glucose sample of Section 310.07, was found to be a highly pure  $\alpha$ -D glucose, lacking contamination from other monosaccharides.

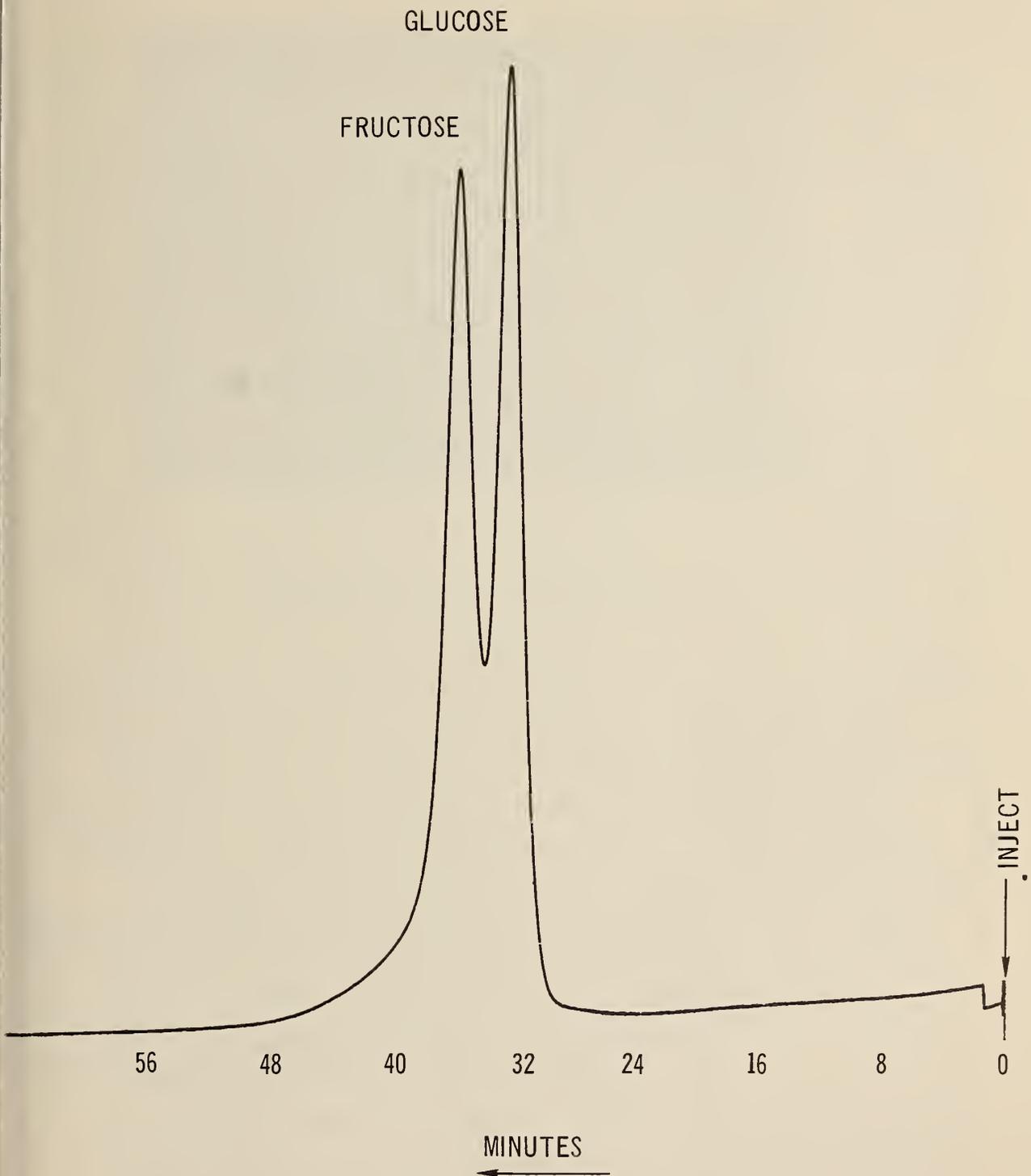


Figure 41. Ion exchange chromatography of glucose.

## 2. Other

a. Organic Dyes. A broad investigation has been in progress by the Analytical Coordination Chemistry Section under Dr. O. Menis to determine a dye standard having a broad and constant absorption useful for the calibration of spectrophotometers. A candidate dye (Neolan Black) was obtained from this Section and subjected to chromatography on Sephadex G-10 using distilled water as the mobile phase under gravity flow. A minimum of five component bands were observed using 0.1 ml of a 10 percent aqueous solution of the dye.

## D. Polyaromatic Hydrocarbons

Polyaromatic hydrocarbons (PAH) occur in the air as environmental toxins produced from the combustion of organic materials such as coal and gasoline. Some of these substances have been shown to be carcinogenic to experimental animals. The analytical procedures which are usually employed include column, thin-layer, and paper chromatography for separations, and fluorescence and UV spectrometry for measurement, using the fractionated crude benzene extract of a particulate filter from a high volume air sampler. An example of contaminated vs. clean air sampled outside and inside a clean room facility is shown in Figure 42. The clean room air filter represents particles greater than  $0.2 \mu\text{m}$  in  $11 \text{ m}^3$  of air sampled at 8.4 l/min. The contaminated filter pad shows such particulates in  $15 \text{ m}^3$  of air sampled at 10 l/min outside the clean room enclosure. For further details, reference is made to Stern [17] (esp. Chap. 20). We successfully chromatographed chrysene, 3,4-benzopyrene, and 1,12-benzoperylene on a pressurized liquid chromatography system using an alumina column, and ether-cyclohexane mixtures as the mobile phase in a procedure similar to that used by NAPCA. Sensitivity limits of these compounds approach  $0.1 \mu\text{g}$  using a UV detector at 254 nm. It was observed that an alumina column of controlled activity (water content) was inappropriate for a continuous dynamic system which could

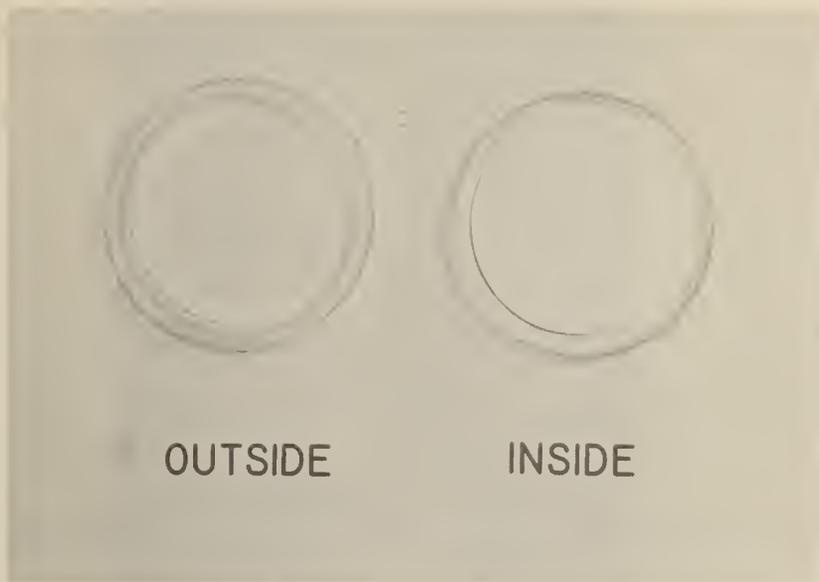


Figure 42. Collection of air borne particles in and outside a clean room facility.

be employed over long periods of time. A column of cellulose formylate gave limiting results. While gas chromatography has shown some degree of promise, no continuous chromatographic system is presently functioning for the complete separation and measurement of all PAH compounds found in air particulates. The outcome of our preliminary ventures (including both liquid and gas chromatography) suggest that appropriate conditions demand sufficiently strong and selective PAH-stationary phase interaction energies (eg. via stationary phase  $\pi$ - $\pi^*$  charge transfer complex formation with the aromatic solutes), perhaps coupled to gradient mobile phase elution.

## 5. HIGH PURITY REAGENTS

### A. Container Contamination

The purity of reagents must depend to some extent upon the inertness of their containers. In the case of ultra pure reagents, the enhancement of trace contamination can arise from chemical attack of the reagent upon the container. This factor can be minimized by choosing a suitably inert container material. FEP Teflon and linear polyethylene are used for storing nitric and hydrochloric acids, respectively. Although each of these materials is inert to the contained acid, particulate matter occluded in the container walls presents a possible source of contamination. Rigorous chemical leaching is used to reduce this effect.

Two-liter insulated Teflon bottles were filled with nitric acid to approximately 5/6 of their internal volume, fitted with condensers and maintained at gentle reflux (110 °C) for six days. Following treatment, the bottles were steamed out for two days, washed, and then dried overnight at 130 °C under vacuum. The nitric acid leaching system is shown in Figure 43.

Polyethylene bottles (500 ml) were refluxed (70 °C) for five days in a 12-liter round-bottomed flask with concentrated hydrochloric acid. The bottles were then steamed out for one day to remove any water-soluble materials in the container matrix, and finally vacuum dried overnight at 55 °C. Since steaming-out periods of at least 24 hours were used, a semi-automatic system was devised using each inverted bottle as a condenser. As illustrated in Figure 44, the inner tube allows steam to flow into the bottle. The inverted bell jar catches the condensate, which is returned to the boiler through the side arm, that also functions as a water trap to prevent loss of steam through it.

A series of independent experiments was carried out to investigate the nature of extraction for and the contaminants removed from FEP Teflon containers. Pieces were cut out of



Figure 43. Nitric acid leaching of teflon containers.

Teflon bottles, water washed, and refluxed for two days in a 3:1 concentrated nitric:hydrochloric acid mixture, followed by reflux for three days in concentrated nitric acid. The presence of hydrochloric acid served to remove entrained metal oxides from the container wall. The leached Teflon pieces were then immersed in boiling water for three days, which was changed daily. The samples were finally dried under vacuum. The removal of a particle 390  $\mu\text{m}$  from the surface of the wall is shown in the series of photomicrographs in Figure 45 together with the phenomenon of flower cavity production. The concentration of particle contaminants in the FEP Teflon containers was observed to be  $1-2 \times 10^4$  particles per  $\text{cm}^3$  of Teflon. Leaching periods of up to five days were required to remove



Figure 44. System for steaming out polyethylene bottles.

large particles (200  $\mu\text{m}$ ). The examination of particle composition by the laser microprobe revealed the presence of Fe, Ti, Ni, Cr, Na, Ca and Si. It appears that the contamination is introduced during the manufacture of FEP Teflon bottles from stainless steel and a talc. The Teflon pieces became opaque after drying of the leached samples. The infrared absorption spectrum of the Teflon prior to and following leaching however, was unchanged.

#### B. Light Scattering Studies

The presence of particulate matter suspended in high purity reagents presents a common kind of contamination. Studies were



START



1 DAY



2 DAYS



3 DAYS

Figure 45. Leaching of a contaminant from the wall of an FEP Teflon container with 3:1  $\text{HNO}_3:\text{HCl}$ .

initiated to evaluate the efficacy of the removal of particulate matter by means of ultrafiltration. A microturbidimeter described previously [1] was used to measure the efficiencies of particle removal.

Standard suspensions of 10 ppm of 0.500  $\mu\text{m}$  and 0.357  $\mu\text{m}$  latex spheres in distilled water previously filtered through a 0.10  $\mu\text{m}$  pore filter were used as feed solutions. The percent removal of the latex spheres by various filters is shown in Table 5. Prior to use, the filters were precleaned by washing with distilled water, which had previously been passed through a 0.10  $\mu\text{m}$  filter, until the filtrates indicated 0.00 ppm particulate concentration in the microturbidimeter.

A second set of experiments was conducted with inorganic salt solutions prepared from fresh reagent bottles and distilled prefiltered water. The level of particulates (ppm) in the salt solutions prior to and following ultrafiltration through a 0.10  $\mu\text{m}$  filter was determined in the microturbidimeter. The results are given in Table 6.

The data indicate that quantitative removal of solution particulate matter is attainable by ultrafiltration when a sufficiently small pore diameter filter is used, and that the pore diameters cited for commercial ultrafilters are usually nominally accurate. Spurry, *et. al.* [18] have investigated the mean radius of Nucleopore filters by electron microscopy and reported the nominal pore radius to be approximately 8 percent larger than the actual mean radius.

### C. Pooled Reagents

Different lots of commercially obtained ultra pure reagents tend to present a bothersome problem due to the corresponding variations in the impurities. This imposes a time-consuming need for separate blank analyses for each reagent. By contrast, a bulk lot of a given reagent implies a more stable situation.

We have obtained 10 liter batch quantities of perchloric, nitric and hydrochloric acids of ultra pure quality. The first

Table 5. Removal efficiency of latex spheres by ultrafiltration.

| Type      | Filters                     |  | Latex spheres |                        | Filtrate conc. (ppm) | Percent removal |
|-----------|-----------------------------|--|---------------|------------------------|----------------------|-----------------|
|           | Pore size ( $\mu\text{m}$ ) |  | Conc. (ppm)   | Size ( $\mu\text{m}$ ) |                      |                 |
| Cellulose | 0.45                        |  | 10            | 0.500                  | 0                    | 100             |
| Cellulose | 0.45                        |  | 10            | 0.357                  | 0.45                 | 95.5            |
| Nuclepore | 0.50                        |  | 10            | 0.500                  | 0                    | 100             |
| Nuclepore | 0.50                        |  | 10            | 0.357                  | 5.0                  | 50              |
| Solvinert | 0.50                        |  | 10            | 0.500                  | 0                    | 100             |
| Solvinert | 0.50                        |  | 10            | 0.357                  | 0.51                 | 94.9            |
| Gelman    | 0.45                        |  | 10            | 0.500                  | 0                    | 100             |
| Gelman    | 0.45                        |  | 10            | 0.357                  | 0.02                 | 99.8            |

Table 6. Removal of particulate matter from inorganic salt solutions.

| Salt                                 | Salt concentration (%) | Measured concentration of particles in salt solutions (ppm) | Particle concentration <sup>a</sup> after filtration (ppm) |
|--------------------------------------|------------------------|---|--|
| CaCl <sub>2</sub>                    | 10                     | 1.58  | 0  |
| Pb(NO <sub>3</sub> ) <sub>2</sub>    | 10                     | 0.13  | 0  |
| Na <sub>2</sub> SO <sub>4</sub>      | 10                     | 0.10  | <0.01  |
| ZnCl <sub>2</sub>                    | 10                     | 0.08  | <0.01  |
| NH <sub>4</sub> Cl                   | 10                     | 0.05  | <0.01  |
| MgCl <sub>2</sub>                    | 10                     | 0.03  | 0  |
| NaHSO <sub>4</sub>                   | 10                     | 0.10  | <0.01  |
| HgNO <sub>3</sub>                    | 10                     | 1.68  | <0.01  |
| NaF                                  | 5                      | 0.17  | 0  |
| AlCl <sub>3</sub>                    | 10                     | 0.08  | 0  |
| Mg(CH <sub>3</sub> COO) <sub>2</sub> | 10                     | 0.10  | 0  |
| Co(NO <sub>3</sub> ) <sub>2</sub>    | 10                     | 0.06  | 0  |
| AgNO <sub>3</sub>                    | 10                     | 0.08  | <0.01  |
| KI                                   | 10                     | 0.12  | 0  |
| KClO <sub>3</sub>                    | 3                      | 0.05  | 0  |

<sup>a</sup>Filtered through a 0.1 μm pore diameter filter.

two acids were packaged in FEP Teflon bottles, while the latter was stored in linear polyethylene containers (see Section 5.A.). The reagents are stored in a chamber maintained at  $-40\text{ }^{\circ}\text{C}$  (Figure 46), affording a projected reagent life of 18 months.



Figure 46. Storage of ultra pure acids at  $-40\text{ }^{\circ}\text{C}$ .

We have also prepared bulk quantities of high purity ammonia solutions from 99.999 percent pure  $\text{NH}_3$  gas. During preparation, candidate impurities in the ammonia gas, such as carbon dioxide, organic vapors, and particulate matter, were respectively removed by passing the gas stream over solid potassium hydroxide, activated charcoal, and through a  $5\text{ }\mu\text{m}$  Teflon filter (Figure 47). To avoid contamination



Figure 47. Removal of trace impurities from 99.999 percent ammonia gas.

from the flow system, the polyethylene tubing, traps, and adsorption container were thoroughly leached with 10 M HCl, EDTA in dilute  $\text{NH}_4\text{OH}$  solution, and steamed out prior to usage.

#### D. Reagent Bank

Our Section supplies, on a "self-service" basis, large quantities of ultra pure reagents to chemists in the Analytical Chemistry Division, either in the form of single commercially packaged units or in specially prepared bulk lots. For all reagents in the bank, decomposition is retarded by storage at temperatures as low as  $-40\text{ }^\circ\text{C}$ . The 150 units dispensed during fiscal 1969 (Table 7) covered 15 separate reagents and represented an increase of 3 percent over that of the previous year. The fact that 90 percent of the units represented either common acids or ammonia solution indicates the need for bulk quantity availability of these reagents.

Table 7. Ultra pure reagents dispensed in fiscal year 1970.

| <u>Acids</u>                   |    | <u>Bases</u>                  |    |
|--------------------------------|----|-------------------------------|----|
| CH <sub>3</sub> COOH           | 2  | NH <sub>4</sub> OH            | 25 |
| HCl                            | 26 | NaOH                          | 2  |
| HF                             | 9  |                               |    |
| HI                             | 1  | <u>Redox</u>                  |    |
| HNO <sub>3</sub>               | 30 | Br <sub>2</sub>               | 1  |
| HClO <sub>4</sub>              | 36 |                               |    |
| H <sub>2</sub> SO <sub>4</sub> | 10 | <u>Buffer Salts</u>           |    |
|                                |    | CH <sub>3</sub> COONa         | 3  |
| <u>Salts</u>                   |    | <u>Acid Anhydrides</u>        |    |
| NaCl                           | 1  | P <sub>2</sub> O <sub>5</sub> | 2  |
| BaCl <sub>2</sub>              | 1  |                               |    |
| NaF                            | 1  |                               |    |

## 6. PERSONNEL AND ACTIVITIES

### A. Personnel Listing

#### Separation and Purification Section

David H. Freeman, Section Chief  
Janice M. Hurst, Secretary

#### Chromatography

Delmo Enagonio  
Walter Zielinski  
Patti Byrne (summer student)

#### Ion Exchange Microstandards

Gerald Sleater

#### Mass Spectrometry

William Dorko

#### Network Structures

Rosalie Angeles (Guest Worker)

#### Particle Metrology

Herbert Dixon

#### Ultra-pure Reagents

Edwin Kuehner

#### Youth Opportunity Corps

Linda Gant  
Jennifer Jones

### B. Talks

1. D. H. Freeman, W. L. Zielinski, Jr., and W. F. Rittner, "Recognition of Crosslinking in the Infrared Spectra of Poly(styrene-divinylbenzene)," International Conference on Ion Exchange in the Process Industries, London, England. July 16, 1969.

2. D. H. Freeman, W. L. Zielinski, Jr., and W. F. Rittner, "Characterization of Ion Exchange Networks: Theory, Microscopy, and Spectroscopy," Gordon Research Conference (Ion Exchange), Meriden, New Hampshire. August 18, 1969.
3. D. H. Freeman, "Ion Exchange Chromatography," 1969 Symposium on Liquid Chromatography, Montreal, Canada. August 25, 1969.
4. D. H. Freeman, V. M. Story, and W. L. Zielinski, Jr., "Ion Exchange Networks: Chromatography and Heterogeneity," 158th ACS National Meeting, New York, New York. September 11, 1969.
5. D. H. Freeman, "Sub-microgram and Sub-nanogram Standards for Chemistry," Anachem Conference, Detroit, Michigan. September 18, 1969.
6. D. H. Freeman, "Ion Exchange Networks: Their Characterization and Applications as Sub-Picogram Standards," Northeastern University, Boston, Massachusetts. October 20, 1969.
7. D. H. Freeman, "Ion Exchange Network Characterization," Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio. March 2, 1970.
8. D. H. Freeman, "Sub-Microgram Encapsulation Chemistry," Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio. March 2, 1970.
9. D. H. Freeman, "Selectivity and Resolution in Gel - HPLC," Washington Chromatography Discussion Group, Washington, D. C. June 11, 1970.
10. W. L. Zielinski, Jr., "Bilirubin Gel Chromatography," Washington Chromatography Discussion Group, Washington, D. C. June 11, 1970.

#### C. Publications

1. R. Angeles, M. H. Aldridge, D. H. Freeman, and L. A. Wall, "Cryodegradation of Polyelectrolyte Networks," J. Poly. Sci., Part B Polymer Letters, Vol. 7, 8, p. 609 (1969).
2. D. H. Freeman, Book Review: "Ion Exchange" Volume 2 (to be published).

3. D. H. Freeman, L. A. Currie, E. C. Kuehner, H. D. Dixon, and R. A. Paulson, "The Development and Characterization of Ion Exchange Beads as Microstandards," Anal. Chem. 42, p. 203-209 (1970).
4. D. H. Freeman and W. L. Zielinski, Jr., "Separation and Purification Section: Summary of Activities July 1968 to June 1969," NBS Technical Note 509, (1970).
5. D. H. Freeman and G. Schmuckler, "Homogeneous Ion Exchangers as Microstandards," XXXIX Conference of the Israel Chemical Society, Jerusalem, Israel, 9/29-10/1/69, p. 174 (1969).
6. W. L. Zielinski, Jr., D. H. Freeman, D. E. Martire, and L. C. Chow, "Gas Chromatography and Thermodynamics of Divinylbenzene Separations on 4,4'-Dihexoxyazoxybenzene Liquid Crystal," Anal. Chem. 42, p. 176-180 (1970).
7. D. H. Freeman, S. Goldstein, and G. Schmuckler, "Homogeneous Sulfonation of Styrene-Divinylbenzene Copolymers with Oleum in Organic Solvents," Israel J. Chem. 7, No. 6 (1969).

## 7. REFERENCES

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manuscript submitted for publication.
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